EFFECTS OF ALLEY CROPPING SYSTEMS ON YIELD AND NUTRITION OF FORAGE CROPS IN SASKATCHEWAN

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ABSTRACT

The agroforestry practice of establishing shelterbelts and/or windbreaks composed of tree and shrub species that include buffaloberry (*Shepherdia argentea* Nutt.), caragana (*Caragana arborescens* Lam.) and sea buckthorn (*Hippophae rhamnoides* L.) is widespread within Saskatchewan. Shelterbelts play major roles in reducing wind speed, trapping snow, improving land-use efficiency and increasing economic returns. However, the practice of alley cropping within Saskatchewan is not popular. Also, apart from the protective roles the tree species offer in shelterbelts, some species have atmospheric nitrogen (N_2)-fixation capabilities through biological nitrogen fixation (BNF) that are potentially important. The simultaneous integration of trees and crops on the same land management unit may lead to competition between crops and trees for growth resources such as nutrients, soil moisture and incoming radiation, the latter leading to limited access of light for understory crops. Understanding the contributions of the trees in supplying nitrogen (N) through BNF and in modifying microclimatic conditions in the alleyways would generate information needed to know their impacts on yield and nutrition of associated crops. In order to assess the contribution of the tree species in supplying N and minimizing interspecific competition while maximizing the benefits of tree-based intercropping systems, the thesis quantified the BNF capabilities of each species under greenhouse conditions using ^{15}N dilution techniques and assessed how much of the fixed N_2 is transferred to associated triticale (*Triticale hexaploide* Lart.) and oats (*Avena sativa* L.) under field conditions. Growth and yield of oats was also studied by measuring photosynthetically active radiation (PAR) and soil moisture in a Manitoba maple (*Acer negundo* var. *negundo* L.) -oats alley cropping system at Indian Head, SK. The BNF results showed that each of the test species fixes a substantial amount of N and there was a high transfer of N to associated triticale and oats. Results from the interspecific

interaction study also showed that soil moisture was the primary factor affecting oats yields followed by light, with the south-lying oat plants affected more than north-lying. It can be concluded that alley cropping systems can be a practical and beneficial agroforestry practice within Saskatchewan. However, the distance between tree rows should be wide enough to permit farm machinery operations.

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1.0 General Introduction

The World Agroforestry Centre (WAC) defines agroforestry as "a dynamic, ecologically-based, natural resources management system that, through the integration of trees on farms and in agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land-users at all levels" (www.icraf.cgiar.org). In the American and Canadian perspectives, agroforestry is synonymous to the practice of intensive land-use management that optimizes the socio-economic, physical, biological and ecological benefits from biophysical interactions that result when trees and/or shrubs are deliberately combined with crops and/or livestock (Gold and Garrett, 2009). The integration of trees and crops in one land management unit leads to a more diverse system with multiple ecosystem functions including supply of the 4 F's (i.e. food, fuelwood, fodder and fibre), medicines, storage of carbon, and conservation of soil and biodiversity (Sileshi et al., 2007; Nair et al., 2008). Agroforestry practices that are currently being researched in North America include shelterbelts and windbreaks, silvopastoral systems, forest farming systems, integrated riparian forest systems, and alley cropping (Gordon and Newman, 1997; Thevathasan et al., 2004).

Alley cropping involves planting of crops or pasture in the alleyways between widely spaced rows of trees, and is one agroforestry system identified for biomass supply within the Prairie region in a recent survey (Marchand and Masse, 2008). When an alley cropping system is properly designed and managed, it will present tangible benefits to farmers that would promote a land management option with greater long-term productivity and sustainability than conventional agriculture (Thevathasan et al., 2004).

Despite the long-term practice of alley cropping around the globe for shelter, wood, browse, fruit or nuts, especially in Europe and in the U. S., they are largely absent from the agricultural landscape of Canada (Bergeron et al., 2011). This may be largely due to the highly mechanized monocropping agriculture, technological and demographic changes (Kort et al., 2009). In contrast to the form of tropical alley cropping, temperate alley cropping has evolved to mean cropping the alleyways between row of trees of varied width grown to maturity without repeatedly coppicing for mulch or green manure (Gillespie et al., 2000). Hence, the key ecological principle to nutrient management and supply in temperate alley cropping systems is to enhance nutrient recycling through different biogeochemical processes including biological nitrogen fixation (BNF). This is achieved through the potentials of the tree or shrub species to fix atmospheric N_2 to replenish soil fertility by supplying plant available nitrogen (N). The fixed N_2 can become available to associated crops via N transfer mechanisms by direct or indirect pathways either belowground or aboveground (Jensen, 1996). This can reduce the input of inorganic N fertilizers, minimizing the incidence of nitrous oxide (N_2O) emissions, and soil and groundwater contamination through nitrate (NO_3^-) leaching (Thevathasan and Gordon, 2004; Dougherty et al., 2009; Santi et al., 2013).

In addition to soil nutrient replenishment through N addition, agroforestry tree or shrub species interact with associated crops leading to the modification of microclimatic conditions such as soil moisture, light and temperature in the alleyways. These interactions impact either positively or negatively on the yield and nutrition of the associated crops. Evidence from the U.S. and eastern Canada showed positive tree-crop interactions under alley cropping practices (Jose et al., 2004; Clinch et al., 2009; Garrett, 2009; Rivest et al., 2009).

Within the Canadian prairies, little is known about how alley cropping impacts on soil moisture, light and temperature in the alleyways to influence crop yield and nutrition. Also, despite the potential of N_2-fixing shrub species such as buffaloberry (*Shepherdia argentea* Nutt.), caragana (*Caragana arborescens* Lam.) and sea buckthorn (*Hippophae rhamnoides* L.) in some land-use systems within the prairie eco-region in reducing wind speed and trapping snow, there is inadequate information on their N_2-fixation capabilities. Understanding the contributions of the trees or shrubs in nutrient replenishing through BNF and their potentials in modifying microclimatic conditions in the alleyways will generate information needed to know their impacts on yield and nutrition within the systems they are used in.

The broad aim of the research is to examine the impacts of trees in alley cropping systems on yield and nutrition of forage crops with specific objectives to 1) quantify N_2-fixation in buffaloberry, caragana and sea buckthorn using ^{15}N dilution techniques 2) determine the percentage of N transfer and the amounts of N transferred to triticale (*Triticale hexaploide* Lart.) and oats (*Avena sativa* L.) from caragana via various belowground mechanisms 3) determine the impacts of modified soil moisture and light within the alleyways on the yield and nutrition of oats.

The thesis is divided into seven related chapters. Chapter 1 introduces the thesis and gives the research objectives. The emphasis is on alley cropping as an agroforestry practice, major tree-crop interactions in alley cropping systems and N supply through BNF and subsequent transfer to associated forage crops. Chapter 2 reviews pertinent literature on the mechanisms of nutrient management and movement in alley cropping systems with emphasis on BNF and methods for estimation, and the factors that affect the fixation process. Literature addressing the practice of agroforestry within the Canadian Prairies is also reviewed with the various tree-crop interactions in typical alley cropping systems as the main focus. The chapter closes with the various

determinants of forage quality. The next three chapters describe the experimental components to achieve the objectives of this thesis. Each chapter begins with a preface, an abstract and contains specific review of literature within the introduction to set the context for the work along with an independent materials and methods sections and ends with separate results and discussion sections. Chapter 3 presents a greenhouse study quantifying the N_2 fixation capabilities of the agroforestry tree or shrub species considered in the research. Chapter 4 premising from the fact that the test species are N_2-fixing plants evaluates and presents results on the percentage of fixed N_2 that is transferred to associated forage crops from the caragana row. Chapter 5 analyzes the various tree-crop interaction mechanisms between Manitoba maple (*Acer negundo* var. *negundo* L.) and oats. Chapter 6 is a synthesis of results from the three preceding chapters (3, 4 and 5) with major conclusions and implications of the research and areas for consideration in future research presented. Chapter 7 is a compilation of all the literature consulted.

2.0 LITERATURE REVIEW

2.1 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is the reduction of atmospheric nitrogen (N_2) to a biologically useful form of nitrogen (N) through symbiotic association between N_2-fixing plants and microbes. The process can occur 1) between legumes and bacterium-*Rhizobium*, including *Azorhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhozobium* (Gualtieri and Bisseling, 2000) 2) between dicotyledonous actinorhizal plants and a Gram-positive, branching and filamentous actinomycetes-*Frankia* (Benson and Silvester, 1993) 3) between a non-legume tropical tree *Parasponia* and Gram-negative, unicellular rhizobia (Appleby et al., 1983) and 4) by free-living organisms (i.e. diazotrophs), though not efficient and significant in intercropping settings such as alley cropping (Stern, 1993). Nitrogen-fixing bacteria in symbiosis with plants play a more significant role in agriculture systems than free-living bacteria as they fix larger amounts of N_2 (Ishizuka, 1992). In temperate climates, N_2 fixation activity in actinorhizal plants can reach up to 300 kg N $ha^{-1}yr^{-1}$ similar to legume plants (Wheeler and Miller, 1990). However, generally, the symbiotic relationship between actinorhizal plants and *Frankia* is less efficient N_2 fixation compared to that of legume-*Rhizobium* symbiosis (Vershinina et al., 2010).

Globally, BNF contributes about 100 to 260 Tg N yr^{-1} (Paul, 1988; Cleveland et al., 1999). The BNF process supplies N to agricultural systems at a rate of between 90 to 140 Tg N yr^{-1} compared to industrial N fixation which supplies N at a rate of between 65 to 83 Tg N yr^{-1} (Paul, 1988; Jenkinson, 2001). Herridge et al. (2008) estimated the annual inputs of N_2 fixation by pasture and fodder legumes to be 12-25 Tg and an overall input of 50-70 Tg N fixed biologically in agricultural systems. This shows that the BNF process can provide an alternative source of N to

inorganic N fertilizers thereby helping to replenish N losses in agricultural systems (Hairiah et al., 2000).

2.1.1 Nodule formation

The BNF process is catalyzed by the nitrogenase enzyme. This enzyme is made up of two main components; the Molybdo-Ferro (Mo-Fe) component-dinitrogenase which is the site for N_2-reduction and an iron-protein or iron-sulfide-protein component which supplies electrons to the dinitrogenase for the N_2 reduction (Giller, 2001). The enzyme is similar in constituents among all N_2-fixing organisms except *Azotobacter* spp. which either does not contain Molybdenum (Mo) in the co-factor or has Vanadium in place of the Mo (Rubio and Ludden, 2005; Newton, 2007). The enzyme complex is sensitive to oxygen (O_2) and its components become deactivated at elevated O_2 concentration. In legume-rhizobia systems, O_2 is excluded from the system by using the cortex to act as a physical barrier to O_2 diffusion and by binding the O_2 to legheamoglobin and transported to respiratory sites (Witty and Minchin, 1990).

Nodulation is a multi-step process that involves specific plant and microbial gene expressions. Nodulation and nodule formation precede the fixation process. Nodule formation is preceded by root hair infection which has several steps. The process begins with the release of flavonoids from roots of the host plants which elicit the production of nodulation (*nod*) factors by the microbe. When the *nod* factor is sensed by the root, a number of biochemical and morphological changes occur leading to nodule formation. The nodule formation pathway is different in legumes and actinorhizal plants. In legumes, the process starts after the recognition of the *Rhizobium* by the host plant on the legume rhizoplane with rhizobial adherence to root hairs causing root-hair curling, infection of the root-hair and transformation of vegetative bacteria into enlarged bacteriods which fix N_2 (Gualtieri and Bisseling, 2000).

In actinorhizal plants, two infection pathways exist; the root-hair infection (RHI) which occurs in the genera *Alnus*, *Casuarina*, *Comptonia* and *Myrica* and intercellular penetration (IP) in the genus *Elaeagnus* (Berry, 1994). The RHI is similar to the process in legumes and is entirely intracellular (Miller and Baker, 1985). However, the IP is different due to the fact that host species have few root hairs, making the process entirely intercellular. The IP process starts with the *Frankia* colonizing the root surface and penetrating the cuticle of the epidermal cells. The hyphae of the *Frankia* then penetrate the middle lamella between two epidermal cells and gain access into the intercellular spaces of the root. Their filaments colonize intercellular space of the cortex and become encapsulated endophytes only after crossing the nodule protoperiderm (Miller and Baker, 1985).

2.1.2 Estimation of biological nitrogen fixation

The aim of quantifying BNF in leguminous and actinorhizal species is to identify the best species which can be integrated into cropping systems (Samba et al., 2002). The methods for quantifying N_2-fixation have been reviewed (e.g., Peoples et al., 1989; Giller, 2001; Murray et al., 2008) and each has its advantages and disadvantages. These methods can be grouped into three distinct groups: The first group estimates N_2-fixation as the net increase in total N of a plant-soil system (e.g., N balance method). The second method separates plant N into what is taken from the soil and that taken from the atmosphere (e.g., N difference, ^{15}N natural abundance, ^{15}N isotope dilution and ureide methods) and the third method measures the activity of the nitrogenase enzyme (e.g., acetylene reduction and hydrogen evolution methods) (Murray et al., 2008).

Some of the methods, however, provide only short-term estimates and hence do not meet the requirements for time-integrated measurements (e.g., acetylene reduction assays and ureide techniques). For example, the ureide technique exploits the fact that many of the tropical legumes

of agronomic significance (e.g. soybean [*Glycine max*], common bean [*Phaseolus vulgaris*] and *Desmodium* spp.) of the family *Leguminosae* produce allantoin and allantoic acid (ureide) in their shoots and nodules as the products of N_2 fixation (Herridge et al., 2008). This therefore restricts its use to only few leguminous plants.

These limitations strongly favour the use of methods that account for temporal variations in order to give realistic estimate, hence the ^{15}N isotopic techniques (Giller and Wilson, 1991). The ^{15}N isotopic techniques are time-integrated and provide estimates of BNF irrespective of yield (Peoples et al., 1996; Boddey et al., 2000). They also provide the best means for quantifying N_2-fixation under field conditions (Hardarson and Atkins, 2003). The ^{15}N isotopic techniques are based on the primary principle that both reference plants and N_2-fixing plants use the same N pool, with the same $^{15}N/^{14}N$ ratio during each phase of growth. These methods rely on differences in isotopic composition of different N sources available for plant growth, i.e., soil N, fertilizer N, and atmospheric N_2 (Bergersen and Turner, 1983).

The choice of a non-N_2-fixing reference plant is of vital importance for estimating BNF using the ^{15}N isotope techniques. The fixing species and the reference plant should take their soil N from the same soil horizons, and their pattern of N assimilation over time should be the same. Important characteristics to be considered when selecting reference crops include the rooting pattern, duration of growth and relative N uptake patterns (Peoples et al., 1989; Pate et al., 1994). Despite the use of a single reference species in quantifying N_2 fixation in many studies, it is recommended to use several reference species under both ideal and stress conditions on different sites over the life cycle of the N_2-fixing plants (Boddey et al., 2000).

2.1.2.1 ^{15}N isotope-based techniques

The ^{15}N natural abundance technique has gained acceptance in use in field and experimental plots due to its greater accessibility, high-precision and automated mass spectrometer (Herridge et al., 2008). It does not require pre-treatment of ^{15}N enriched material, therefore imposes minimal disturbance to established agroecosystems and has no problem with non-uniform labeling of trees or shrubs within the rooting zone (Chalk, 1998; Eriksen and Høgh-Jensen, 1998). The ^{15}N natural abundance technique does not reduce the precision of BNF estimates compared to ^{15}N isotope dilution method (Høgh-Jensen and Schjoerring, 1994), however, the accuracy of the BNF estimates is influenced by the degree and uniformity of the ^{15}N abundance in the soil where the crop is growing (Gathumbi et al., 2002). The ^{15}N natural abundance method can account for differences in rooting depth between the fixing and non-fixing reference plants, especially when agroforestry trees or shrubs are involved. However, it has some limitations that limits its use in natural agroecosystems including diversity and complexity of soil available N-pools with varied ^{15}N signatures (Gehring and Vlek, 2004). It is also potentially more susceptible to errors associated with remobilization and recycling of fixed N_2 and isotopic discrimination processes during N transformations in established ecosystems (Handley et al., 1994).

The ^{15}N isotope dilution technique usually involves growing plants in ^{15}N-enriched soil or other growing medium and is appropriate for annual crops and herbaceous N_2 -fixing plants. The technique does not usually require high analytical sensitivity and the potential influence of isotope fractionation processes in the soil and in the N_2 fixation process is reduced (Chalk, 1991; Carranca et al., 1999). However, it has some limitations including disturbance of established agroecosystem due to the application of ^{15}N enriched fertilizer; high costs of the enriched fertilizer making it applicable to a limited area of land and low accuracy in quantifying low levels of symbiotic N_2-

fixation (Hardarson et al., 1988). Another limitation especially in trees is the non-uniformity of the applied ^{15}N fertilizer within the rooting zone (Hardarson et al., 1988).

2.1.3 Factors affecting biological nitrogen fixation

All environmental factors, inherent physiological plant factors (e.g., seasonal production of leaf and fine root biomass, nodule appearance), soil nutrient dynamics and agronomic management practices that have impacts on plant vigour and growth influence the BNF process (Bordeleau and Prévost, 1994; Mohammadi et al., 2012).

Deficiency in soil phosphorus (P) hampers nodulation, nodule formation and N_2 fixation (Labidi et al., 2003). There was a significant increase in nodulation and grain yield in P treatments compared to the control, an indication that P was limiting nodulation and yield (Jemo et al., 2010). In nutrient-poor environments, the absolute availability of soil P more strongly controls rates of BNF than the N: P ratio of the soil (Reed et al., 2007). Inadequate amounts of micronutrients also negatively affect BNF (Sprent and Minchin, 1983).

The soil pH range that favours nodulation is neutral to slightly alkaline, with low pH negatively affecting both microbial survival and root-hair infection, leading to less nodule formation (Tang and Thomson, 1996). This is because at low pH there is bioavailability of toxic elements such as aluminum and manganese and low concentrations of P, molybdenum and calcium (Bordeleau and Prévost, 1994). There was a decrease in nodulation when plants were grown at pH 4.5 compared with those grown at pH 5.8 (Richardson et al., 1988).

Available soil moisture content is also an important parameter controlling BNF. It influences nodulation by forming a thin layer covering the nodules which decreases O_2 diffusion hence deactivating the activities of the nitrogenase enzyme. Excess water facilitates the build-up of carbon dioxide (CO_2), creating an anaerobic condition in the rhizosphere which inhibits nodule

formation. Under this anaerobic condition, ethylene is produced, which even at lower level inhibits nodulation (Sprent and Minchin, 1983; Stevenson et al., 1995).

Adapted young trees on low-fertility soils would nodulate abundantly and fix N_2 actively due to the limited N supply from the soil. In mature tree based-systems, however, N_2-fixation often is slow due to a simultaneous increase in soil N following the recycling of fixed N_2 in litter and prunings (van Kessel et al., 1994; Mohammadi et al., 2012).

The range of temperature within which both nodulation and BNF processes occur is wide and preferably between 20 to 30 ^{o}C or up to 36 ^{o}C (Bordeleau and Prévost, 1994). Elevated temperatures delay the initiation of nodules and nodule development interfering with the structure and function of nodules in temperate legumes. However, in tropical legumes, elevated temperatures affect the overall efficiency of the BNF process (Bordeleau and Prévost, 1994). Effects of temperature on biological processes such as photosynthesis, respiration and transpiration directly or indirectly affect the BNF process. For instance, an increase in temperature increases the rate of respiration, limiting the availability of carbon (C) for the *Rhizobium*, leading to reduced efficiency of BNF. At higher temperatures, a large fraction of the fixed N_2 is not incorporated into cell due to reduced photosynthesis or increased membrane permeability, but released rather (Breitbarth et al., 2006).

Application of N fertilizer is a means of increasing available N in the soil even in N_2-fixing-based systems. Significant yield response was observed in nodulated soybean (*Glycine max* [L.] Merr.) due to frequent N addition when N_2-fixation could not meet its N demand (Thies et al., 1995). Low levels of combined N applied at germination stimulate plant growth leading to increased nodulation, especially under conditions that delay nodule development or adverse environmental conditions (Atkins, 1983). Though early season N-deficiency hampers crop growth

and efficiency of the nodulation process, early application of N fertilizer results in temporary suppression of nodule establishment and activity (Hungria et al., 2005). Added N reduces the supply of carbohydrates to underground plant organs, with nodules affected more than roots (Pate, 1977). Also, addition of N (i.e., NO_3^- or NH_4^+) fertilizer to nodulated legumes has negative effects on nodule formation and structure and on BNF in general. Nitrate (NO_3^-) has three main effects on the symbiotic association between legumes and rhizobia: 1) its role during the root infection process 2) on nitrogenase activity and 3) on the ratio of nodule dry mass to whole plant biomass (Streeter, 1988). There was an observed antagonism between BNF in the nodule and NO_3^- concentration in soil solution in soybean (Streeter, 1988) when soil moisture, soil pH and soil temperature were not limiting factors (Bordeleau and Prévost, 1994; Stevenson et al., 1995; Tang and Thomson, 1996). Three hypotheses have been proposed to explain the effects of NO_3^- concentration on BNF: 1) the presence of NO_3^- diverts C to the site of NO_3^- assimilation, depriving the nodules of the requisite C for energy; 2) during NO_3^- reduction, the nitrite (NO_2^-) produced by the bacteriod-cystolic nitrate reductase inhibits the activities of the nitrogenase enzyme; 3) NO_3^- increases the resistance to O_2 diffusion within the nodule (Vessey and Waterer, 1992).

2.2 Mechanisms of Nitrogen Transfer in Alley Cropping Systems

The overuse of inorganic N fertilizers can cause environmental problems through pollution of groundwater and surface water by NO_3^-, and release of nitrous oxide (N_2O) into the atmosphere. It also has cost implications to farmers (Paynel et al., 2008; Fustec et al., 2010). To reduce the aforementioned problems, systems involving mixtures of N_2-fixing plants and non-N_2-fixing plants represent a sustainable alternative and are capable of offering an economically

attractive, ecologically efficient and environmentally-friendly means of introducing N into land-use systems (Paynel et al., 2008), chiefly through the transfer of the fixed N_2.

The main pathways of N transfer from N_2-fixing to non-N_2-fixing plants are the "direct pathway" and "indirect pathway". In the "direct pathway", N is transferred between N_2-fixing plants and non-N_2-fixing plants via common mycorrhizal networks (CMN) interconnecting the root systems of both species (He et al., 2003; Paynel et al., 2008). The "indirect pathway" occurs through the soil medium via N rhizodeposition into the soil followed by uptake by associated crops (Rogers et al., 2001; Høgh-Jensen and Schjoerring, 2001). The indirect pathway mainly occurs through the death of above- and belowground plant tissue and subsequent degradation and turnover in the soil (Ledgard and Steele, 1992) and through N deposited by root exudation by living roots (Paynel et al., 2001). Due to escalating environmental and economic pressures, N transfer from legumes has received particular attention generally in cropping systems such as legume-based pastures and annual crop associations in the last two decades (Høgh-Jensen and Schjoerring, 2000; Chu et al., 2004). However, belowground N transfer from legume trees to associated crops is a poorly quantified N source in agroforestry systems (Khan et al., 2002).

2.3 The Canadian Prairie Eco-region

The Canadian prairie region which spans Manitoba, Saskatchewan and Alberta, is located at the periphery of the agricultural production zone within the Great Plains of North America (Nadler and Bullock, 2011). The region has a cold continental, sub-humid to semiarid climate and the relief consists of typically low-lying valleys and plains sloping eastward. It shares borders with Ontario to the east and British Columbia to the west. The natural vegetation of the region is typically short-grass prairie in the southwest, transitioning to medium and tall grasses in the

northeast, then to aspen parklands, boreal forest and finally to tundra in northern Manitoba (Thevathasan et al., 2012). The region consists of approximately 30 million hectares of crop land that lies between 49° and 60° N latitude and 95° and 120° W longitude (Nadler and Bullock, 2011). Most of the region is dominated principally by Brown, Dark Brown, and Black Chernozemic soils which formerly supported the short, mixed, and tall grass prairies, respectively (Soil Classification Working Group, 1998). While the soil resource remains relatively constant within the region, the weather is subject to erratic variability, making it an unpredictable aspect of agriculture. Mean annual temperatures within the region range between about 2.0 ºC to about 5.5 ºC with monthly mean daily temperatures below 0 ºC from November to March. Soils are frozen for three to five months each year. Precipitation in the region ranges from about 300 to 550 mm annually and about 70 % to 80 % of annual precipitation falls as rain in spring, summer, and fall. Snowmelt usually takes place over more than one month in the spring, with much of the melt taking place in a short, intensive melt period. As a result, snowmelt is the largest and most consistent annual runoff event in most areas of the prairie region. Soils usually have significant soil moisture storage potential as late-winter and early-spring soil moisture levels are usually well below field capacity (Nadler and Bullock, 2011).

2.4 Agroforestry in the Canadian Prairies

Traditionally, agroforestry has its origins in developing nations where high population densities coupled with scarce land resources require that concurrent food and wood production occur on the same land base. In Canada, the modernization of production agriculture in the past 150 years came with its attendant problems including, soil erosion by wind and water, and declining crop production among others (Thevathasan et al., 2012). Agroforestry, a new name for an old set of practices represents a possible solution to some of the problems faced by production

agriculture. Within the Prairie Provinces of western Canada, establishment of shelterbelts and windbreaks on fields, farmyard and on roadside date back to the 1900s. However, following drought, depression and soil erosion in the 1930s, the government of Canada passed the Prairie Farm Rehabilitation Act through which field shelterbelt planting immensely increased (PFRA, 2000). This represents the first form of institutionalized agroforestry practice within the prairies.

The deliberate planting of trees within the prairies was a long-term practice through the tree planting efforts of farmers and the southward spread of aspen poplar (Howe, 1986). The practice was further sustained through the supply of trees to farmers by the federal government throughout the prairies between 1888 and 1899 (Howe, 1986). By 1901, ten and fifteen acres of land were set aside at Brandon and Indian Head, respectively for use by the Forestry Branch of the Department of Interior to begin propagation of tree seedlings (Howe, 1986). In 1902, a permanent nursery was established at Indian Head on 160 acres of land to produce shelterbelt stocks for farmers which was later consolidated into a nursery under the Department of the Interior in 1906 (Howe, 1986). In 1963 the nurseries were transferred to the Prairie Farm shelterbelt Rehabilitation Administration (PFRA) under AAFC which was nationalized and re-named the Agri-Environment Services Branch (AESB) in 1986. In 2012, the AESB was merged with the Research Branch to form the Science and Technology Branch. Due to the discontinuance of the AAFC's prairie shelterbelt program in spring 2012, the last batch of trees under the program was shipped in June 2013.

Despite the termination of the prairie shelterbelt program, other agroforestry practices are still being pursued both at the experimental and landscape levels by the Agroforestry Development Centre (ADC) –Indian Head. For example, in order to explore the impacts of the species planted through the PFRA; this Masters project was funded through the Research Affiliate

Program (RAP) to study the impacts of the trees on the nutrition of associated crops in terms of N supply and microclimatic modifications.

Since the 1990s, the adoption of agroforestry within the prairies has changed due to technological advancement and socio-economic circumstances. For example, the establishment of multiple-row windbreaks has received attention within the region due to intensive swine production. These windbreaks reduce odour and serve as buffers for surrounding residences and urban areas (Thevathasan et al., 2012).

Agroforestry in the recent past has received attention as an alternative land-use management to conventional agriculture practices in the temperate region and within the prairies, due to its efficient use of resources, environmental 'friendliness' and its multipurpose benefits (Jose and Gordon, 2008). This has received further support through research conducted by research institutions and universities. Within Saskatchewan, the Centre for Northern Agroforestry and Afforestation established in 2006, is affiliated with the College of Agriculture and Bioresources, University of Saskatchewan (U. of S.) and conducts research in various aspects of agroforestry. The Saskatchewan Research Council also collaborates with U. of S. scientists and other stakeholders to develop new agroforestry options. In Alberta, the provincial government, Agriculture and Agri-Food Canada (AAFC) and industry support the Agroforestry and Woodlot Extension Service with the aim of conducting research and developing projects related to agroforestry and transferring technology to landowners. In Manitoba, the provincial agriculture department provides technical support in agroforestry to farmers and other stakeholders (Thevathasan et al., 2012).

Currently, a number of agroforestry practices are implemented within the prairie eco-region: Riparian buffers are implemented on small-scale and are promoted primarily as a means of

ensuring water quality from the high risk of N and P runoff from cropping activities. Silvopasture is commonly practised through forest grazing in aspen forest though not intensively managed. There is wild-crafting of mushrooms and other non-timber forest products (NTFPs) as a form of forest farming, even though it is not done as a popular practice. Alley cropping sites are few and the practice is confined to high-value crops (Thevathasan et al., 2012). To make agroforestry practices such as alley cropping acceptable to farmers, there is the need to show that biophysical interactions are beneficial per unit area of land in an intercropping setting compared to a monoculture setting (Jose and Gordon, 2008). Due to differences in climate, topography, soils, plant and animal species, not a single optimal agroforestry practice design is known for both farmers and researchers to adopt. Therefore, any agroforestry practice should be designed to fit the particular context of farmers and landowners (Jose and Gordon, 2008).

2.5 Interactions in Agroforestry Systems

Interactions in agroforestry in general, occur at the tree-crop and/or tree-animal interface and are the influence of one component of the system on the performance of the other components of the system and on the overall system itself (Nair, 1993). The interactions may either occur belowground or aboveground and can be detrimental or beneficial. Understanding of the biophysical interactions and the allocation of growth resources is vital for the development of socially acceptable, environmentally friendly, economically viable, and ecologically sound agroforestry systems (Jose et al., 2009). Also, exploitation of positive interactions between the woody and non-woody components and the minimization of negative interactions is the key to the success of agroforestry systems. Therefore, agroforestry systems should be designed such that the physiological needs for a particular resource are varied either spatially or temporally for the

different components. Under this situation, there would not be negative impacts of one component of the system on other (s). The system may accrue a total productivity higher than the combined production of the various components grown separately on an equal land area (Cannell et al., 1996; Wojtkowski, 1998).

2.5.1 Aboveground interactions

The integration of trees or shrubs in an agroforestry system can elevate the amount of shading that plants experience, especially the understory species compared to growing in a monoculture setting (Jose et al., 2004). Both the fraction of the incident photosynthetically active radiation (PAR) plants intercept and the ability to convert it into energy through photosynthesis are vital factors in plant biomass growth (Ong, 1996). This biomass growth is influenced by a number of factors such as temperature, CO_2 level, aspect, time of the day, photosynthetic pathway (C3 vs. C4), plant age and height, leaf area and angle, canopy structure, transmission and reflectance characteristics and species combination (Brenner, 1996; Kozlowski and Pallardy, 1997). Shading by tree species is a factor contributing to reduced crop yield (Gillespie et al., 2000; Reynolds et al., 2007). Plants with C3 photosynthetic pathway are thought to be shade tolerant compared to plants with C4 pathway. Results from a study showed that as PAR increases from complete shade to approximately 25 to 50 % of full sun, there is a corresponding increase in the rate of photosynthesis in C3 plants; however, photosynthesis rate flattened as light becomes available with a corresponding increase in PAR (Reynolds et al., 2007). In contrast to C3 plants, C4 photosynthetic rate did not flattened as PAR increases to full sunlight but rather increases with increasing PAR. This behaviour relates to how C3 and C4 fix CO_2 (Kozlowski and Pallardy, 1997; Lambers et al., 1998). Shading, in addition to the negative effects it confers on yield of understory species, can also have positive and neutral effects on the yield of understory

vegetation. For example, treatment of below 80 % shade resulted in greater crude protein values of most of cold-season forage grasses compared to the full sun treatment (Lin et al., 2001). This may be attributed to reduced stress due to reduced evaporative loss compared to the full sun treatment. Also, irrespective of shading, no apparent yield reduction was observed when belowground competition for nutrients and moisture was controlled by polythene root barrier (Gillespie et al., 2000). As opposed to the expected yield decrease in maize (*Zea mays* L.), there were no effects of shading on maize yield in both a black walnut (*Juglans nigra* L.) ―maize and red oak (*Quercus rubra* L.) ―maize alley cropping systems in Indiana, USA (Gillespie et al., 2000).

Temperature modification within tree-based intercropping is apparent due to the effects of the upper story canopy on the understory vegetation. Under higher temperatures, there is evaporative heat loss from the understory vegetation and from the soil surface which lowers the soil moisture condition and the yield of associated vegetation. In addition to solar radiation, trees can influence the microclimate through the reduction of wind speed, increasing humidity and trapping snow (Jose et al., 2004). This may increase the amount of soil water available to the understory vegetation. In a study conducted in northwestern Florida in a pecan (*Carya illinoensis* K. Koch) ―cotton (*Gossypium hirsutum* L.) alley cropping system, cotton plants under pecan canopy germinated earlier than cotton monoculture. It was concluded that it was due to improved moisture and soil conditions in the intercropping situation (Ramsey and Jose, 2002).

Trees used in alley cropping systems can modify soil structure and the enhance soil water holding capacity. Trees on farmland tend to increase permeability of the soil, stability of aggregates, water-holding capacity, and alter soil temperature regimes, leading to enhanced medium for plant growth (Nair, 1987). Through the network of roots and the interception ability

of tree canopies, soil erosion is minimized by rechanneling of rainfall. Wind patterns are also altered by tree canopies, which tend to be less damaging to soil (del Castillo et al., 1994). Addition of significant amounts of litter also increases the organic matter (OM) content of the soil. This improves the soil physical and chemical properties and also provides cover to the soil. Soil OM increased by 4-7 % in alley cropping systems involving red alder (*Alnus rubra* Bong.) and maize (*Zea mays* L.) compared with a maize monoculture following four years of cropping (Seiter et al., 1999). High OM content increases soil aeration and porosity by reducing compaction, leading to improved overall soil physical properties (del Castillo et al., 1994).

2.5.2 Belowground interactions

In water-limiting environments in temperate zones, alley cropping practice alters the soil water due to the interactions between the trees and associated crops with attendant effects on plant growth and productivity. In temperate alley cropping systems, water is a major limiting factor (Jose et al., 2000a; Miller and Pallardy, 2001). The presence of trees in a land-use system alters the soil water availability with consequences on all associated plants. Deep-rooted trees have relatively higher fine root biomass than agricultural crops, placing them in a favourable position for water uptake compared to neighbouring agricultural crops (Jose et al., 2000a). Due to the differences in the rooting depth of the trees and associated crops, exploitation of water resources may occur at different depths (Wanvestraut et al., 2004). Ultimately, the effects of soil moisture depletion on crops are expressed in the form of lower emergence rates, diminished plant size, and decreased yield (Jose et al., 2000a).

Nutrients are limiting factors in agroforestry systems and in conventional agriculture. However, interspecific competition for nutrients is generally of minor importance to the productivity of systems, except when certain factors interact to increase nutrient competition. For

example, competition for fertilizer N was minimal in a black walnut-maize alley cropping system, since nutrient acquisition was not simultaneous among the components of the system (Jose et al., 2000b). However, availability of water was a factor affecting nutrient competition, as competition for water by tree roots was responsible for a reduction in biomass in intercropped maize, resulting in decreased fertilizer use efficiency (Jose et al., 2000b). Belowground competition for water and nutrients can be controlled by installing root barriers. Cotton plants growing with a barrier treatment were 26 % taller and had 48 % more leaf area by the end of the growing season compared to the cotton plants in the non-barrier treatment in a pecan-cotton alley cropping system in Florida, USA, where competition for water was observed (Wanvestraut et al., 2004). This led to 35 % higher in lint yield in the barrier treatment compared to the non-barrier treatment. Competition for N was observed in a pecan-cotton alley cropping system in northwest Florida, USA, where cotton plants in the barrier treatment had 59 % higher aboveground biomass compared to plants in the non-barrier treatment (Allen et al., 2004). This was attributed to the fact that pecan trees leaf out earlier in the spring and have a high nutrient demand early in the growing season. Soil N was depleted before cotton plants were established later in the growing season.

2.6 General Species Descriptions

2.6.1 Sea buckthorn (*Hippophae rhamnoides* L.)

Sea buckthorn is a shrub that performs well on well-drained, light to medium textured loamy soils. A drought tolerant strain "Indian Summer" developed through the AAFC-Agroforestry Development Centre's Tree Improvement Program, grows well in full sun, and thrives on nutrient poor soils due to its ability to fix atmospheric N_2 (Pollock and Svendsen, 2008). Sea buckthorn occurs is naturally distributed widely throughout temperate zones between latitude 27 º and 69 º N, and longitude 7 º W and 122 º E (Pan et al., 1989), including Russia,

China, Mongolia, Great Britain and northern Europe. It can withstand temperatures from -43 to 40 º C. It has attracted attention during the last decade due to its nutritional and medicinal value (Li and Schroeder, 1996). Sea buckthorn was originally imported to the Morden Research Station, AAFC, Morden, Manitoba, Canada from Russia in 1983 (Davidson et al., 1994). Except in Saskatchewan and Manitoba where it has been used in agroforestry, plantings were limited to ornamental landscapes. Sea buckthorn has an extensive rooting system and can be propagated by suckering; hence it is an ideal plant for preventing soil erosion (Yao and Tigerstedt, 1995). It belongs to the family *Elaeagnaceae* and therefore forms a highly efficient relationship with *Frankia* to convert atmospheric N_2 to a form used for plant nutrition. The *Hippophae* associations contribute between 27 to 179 kg N ha^{-1} yr^{-1} (Baker and Mullin, 1992; Gentili and Huss-Danell, 2002). Studies by Akkermans (1971) found that sea buckthorn can fix up to a maximum of 15 kg N ha^{-1} yr^{-1}. This attribute makes it an ideal species for reclaiming degraded land (Schroeder and Yao, 1995). The ecological importance of sea buckthorn lies mainly in its contribution to available soil N in the form of N rich compounds which are directly deposited into the soil as sloughed root tissues and root exudates. Litter produced by sea buckthorn is also a good source of N because it is labile with low C:N ratio (Domenach et al., 1994). In other actinorhizal plants such as *Alnus*, calculated fixation was about 60 kg N ha^{-1} yr^{-1}, but fixation can reach up to 130 kg N ha^{-1} yr^{-1} in some localities (Akkermans, 1971). An appreciable amount of the fixed N_2 can be transferred from the tree to associated crops via connecting mycorrhizal hyphae (Domenach et al., 1994).

2.6.2 Caragana (*Caragana arborescens* Lam.)

Caragana is a multi-stemmed shrub introduced into Canada from Siberia, with a mature tree average height of between 3 and 5 m (Pollock and Svendsen, 2008). It grows best on well-drained

sites under full sunlight condition. The seed strain ‘Ross’ developed through the AAFC – Agroforestry Development Centre's Tree Improvement Program, is very drought tolerant and cannot withstand prolonged flooding. The genus *Caragana* is composed of approximately 100 species distributed within northern Eurasia from the black sea to southeast Siberia, and southward to eastern and south-western China, Nepal, Afghanistan and Turkmenistan (Zhang et al., 2009). Caragana has a temperate Asian distribution and commonly occurs in arid regions that can reach extremely cold temperatures (Zhang et al., 2009). Species also occur in forests, grasslands, deserts and alpine meadows (Zhang et al., 2009).

It belongs to the family *Leguminosae* and fixes atmospheric N_2 in association with *Rhizobium* (Moukoumi et al., 2012). The shrub thrives on low fertility soils due to its BNF capabilities and high competitive ability. It initiates BNF at temperatures of 3 to 5°C, which is considerably lower than in many other species, contributing to its greater northern hardiness limit than most other studied species (Hensley and Carpenter, 1979; Pollock and Svendsen, 2008). Due to this BNF role, it has been globally cultivated for soil stabilization, and mine and construction site improvement (Meng et al., 2009). However, in North America, it has been planted extensively as shrub buffer strips and shelterbelts on farms (Henderson and Chapman, 2006). Farmers and landowners planted caragana primarily for windbreaks on farms during the 1930s, though it served the purpose as habitat for wildlife and erosion control. The Canadian government provided support to the program by supplying seedlings, financial assistance and publicity (Henderson and Chapman, 2006).

The species is known for its tolerance of many adverse environmental conditions including drought, temperatures to -38 °C, infertile soils, sunny sites, high winds, alkaline soils and saline conditions (Henderson and Chapman, 2006; Martine et al., 2008; USDA NRCS, 2010). This

tolerance for cold and dry sites is almost certainly associated with its much greater spread through North America relative to other adapted species. It is well adapted to climatic conditions in Saskatchewan and is potentially a good source of biomass because it produces substantial amounts of biomass in a short growing season (Marchand and Masse, 2008).

2.6.3 Silver buffaloberry (*Shepherdia argentea* Nutt.)

Silver buffaloberry also known as "the thorny buffaloberry" is a thorny, thicket-forming, dioecious, tree-like plant which grows to height of 1.0 m to 6.0 m. It is an endemic North American species of the family *Elaeagnaceae* (Hayes et al., 1989). The species grows well on moist and well-drained soils. It is drought tolerant and grows well on soils with moderate salt content (Pollock and Svendsen, 2008). It thrives well on sites with either full sunlight or partial shading. Due to its capacity to fix atmospheric N_2 in a symbiotic association with *Frankia*, it thrives well on low-fertility soils (Miller, 2011). Buffaloberry, like other actinorhizal plants has the ability to inhabit soils with low N availability and is often an early successional species after disturbance and can be employed in the revegetation of degraded sites (Huss-Danell, 1997). A study by Hendrickson and Burgess (1989) in southern British Columbia found that buffaloberry (*Shepherdia canadensis* L.) in a 4-year regenerating lodge pole pine stand can fix an annual average N of 0.78 kg N ha^{-1} yr^{-1}.

2.6.4 Choke cherry (*Prunus virginiana var melanocarpa* L.)

Choke cherry is a deciduous shrub of the family *Rosaceae* and has two main recognized varieties; var. *melanocarpa* and var. *demissa* (Cronquist et al., 1997). The species inhabits a range of sites across Canada and the United States. Notwithstanding its habitat in Canada (stretching from Newfoundland in the east to British Columbia in the west), scattered stands can be found as

far north as the southern portion of the Northwest Territories (Little, 1976). In the U.S., it can be found as far as western Texas, southwestern and southern California, though isolated patches can be found in Virginia, Kentucky, North Carolina and Oklahoma (Little, 1976). Choke cherry does not form any symbiotic associations with either *Rhizobium* or *Frankia*, hence does not fix atmospheric N_2 (Pollock and Svendsen, 2008). In this thesis, it is employed as a reference species to quantify N_2 fixation in the test agroforestry species. It is usually found growing in valley bottoms, sheltered slopes, along streams and roads. It thrives on a wide range of soils ranging from Regosols to Chernozems with textures ranging from sandy loams to clays (Pacific Southwest Experiment Station, 2002). It is tolerant of moderately saline soils, but it cannot tolerate poorly drained soils and as such suffers from prolonged flooding. It grows on soils with pH ranging from 3.6 to 7.6. It tolerates partial shade and resprouts from root crowns and rhizomes readily, consequently giving it the capacity to persist under open or forest canopies of moderate densities (Pacific Southwest Experiment Station, 2002).

2.6.5 Manitoba maple (*Acer negundo* var. *negundo* L.)

Manitoba maple (*Acer negundo* L.) also called "box-elder" occurs naturally throughout Saskatchewan and Manitoba and has been planted extensively in Prairie shelterbelts (Kort and Michiels, 1997). It is native to the Prairies of North America and is mostly found in moist soils around streams, lakes and the swamp margins. It is drought tolerant when planted on drier sites (Canada Department of Forestry and Rural Development, 1966). It has gained popularity in use for ornamental and street plantings as well as shelterbelts due its cold-hardiness, drought tolerance with reduced growth rate and early growth characteristics (Kort and Michiels, 1997; Pollock and Svendsen, 2008). When planted in shelterbelts usually at the centre of the tree rows, it confers a

wind protection function to the shelterbelts (Pollock and Svendsen, 2008). Maple has bushy and spreading crowns, reaches a height of between 15 and 20 m with a life span of 60 years (Overton, 1990). Under Canadian Prairie conditions, however, its height at maturity has been reported to be 14 m (PFRA Shelterbelt Centre, 1989).

2.6.6 Triticale (*Triticale hexaploide* Lart.)

Triticale (*X Triticosecale* Wittmack) is a synthetic crop developed by crossing wheat (*Triticum turgidum* L. or *Triticum aestivum* L.) with rye (*Secale cereale* L.). The name 'triticale' is a worldwide crop name derived from the combination of the scientific classification of the two genera involved, which is wheat (*Triticum*) and rye (*Secale*). Triticale cultivars, grown for forage and grain can be grouped into three types namely; spring, winter and intermediate (facultative). The spring types show upright growth and produce much forage in their early growth stage. They are insensitive to photoperiod and have limited tillering. The winter types are generally planted in fall but in some situations they can be planted in spring. They have horizontal growth habit in the early developmental stages. Generally, the winter types have higher forage yield than the spring types due to their long growth cycle. The facultative types are the intermediate between the winter and the spring types (Mergoum et al., 2004; Salmon et al., 2004). Although a relatively new crop, its history dates back to the 1870's when the first cross was attempted in Scotland. Triticale generally combines the high yield and good grain quality of wheat with disease and environmental tolerance of rye. It is a crop suitable for marginal or disease prone environments.

Currently, production is concentrated in Europe with almost 90 % of the world production and approximately three million hectares harvested yearly. Production in the U.S. is approximately four hundred thousand hectares, with the majority of planted hectares used as forage and pastures. In 2009, according to FAO, 15 million tons of triticale grain was harvested in

29 countries across the world (FAO, 2013). Globally, triticale is now a well-established crop and is used for food, feed (for ruminants and non-ruminants), grazed or stored forage and fodder, silage, green-fed and hay (Mergoum et al., 2004). The protein content of triticale, which is higher than wheat, is between 10 % and 20 % on dry weight basis. Diets containing triticale grain are balanced to meet the lysine needs rather than crude protein requirements (Myer, 2002). Modern triticale varieties have comparable energy value to other cereal grains used in mixed diets of beef and dairy cattle, sheep, broilers and laying hens, pigs and its protein content is utilized well (Gursoy and Yilmaz, 2002). Triticale can also act as a soil improver as its roots bind soil particles together thereby reducing the incidence of soil erosion (Salmon et al., 2004).

The AC Ultima spring triticale used in this research, originated from the International Maize and Wheat Improvement Centre (CIMMYT) triticale breeding program. It was introduced in 1993 by the Semiarid Prairie Agricultural Research Centre (SPARC), Research Branch, AAFC, Swift Current, SK, as part of the triticale breeding project (McLeod, 2000). It is well-adapted to the soils of the Canadian Prairies. It has a relatively higher yield, matures faster and has a higher kernel weight compared to AC Copia, Pronghorn and AC Certa cultivars across the Brown and Black soils of the Canadian Prairies (McLeod, 2000). It is grown as a high-yielding feed grain, high quality grain for baked goods and industrial ethanol feedstock (McLeod, 2000).

2.6.7 Pinnacle Oats (*Avena sativa* L.)

Pinnacle oats is a high-yielding, white-hulled oats which is well-suited for the oats growing areas of western Canada and in particular the Black Soil Zone of Manitoba and Saskatchewan based on kernel quality, disease and agronomic data (Mitchell Fetch et al., 2003). It was developed by AAFC, Cereal Research Centre, Winnipeg, MB (Mitchell Fetch et al., 2003). It has good resistance to all of the diseases found within the western part of the Canadian Prairies.

2.7 Determinants of Forage Quality

Forage quality is the capacity of given forage to meet the nutrient needs of the consuming animal (Van Saun, 2006). This can be measured using different indices.

Dry matter (DM) is defined as the non-moisture portion of a feed element. This fraction contains the essential nutrients in given forage. Various feed materials vary widely in their DM. Pastures and liquid feeds have DM values between 10 and 25 %. This portion of a feed is determined by heating a weighed sample of feed in a convection drying oven until a constant weight is reached within 24 to 48 hours. Dry weight is expressed as a ratio to original sample weight (moisture + DM) or converted to a percentage (Van Saun, 2006).

The fibre fraction of forage is characterized by the detergent feed analysis. The portion of forage sample insoluble in neutral detergent is termed neutral detergent fiber (NDF). This contains the principal components of the plant cell wall, namely, hemicellulose, cellulose, and lignin (Van Saun, 2006). As the plant matures leading to an increase in cell wall production, the NDF content also increases. Neutral detergent fibre is a good measure of feed quality and plant maturity. For legume forages, good quality feed is defined as less than 40 % NDF. Neutral detergent fibre in feed above 50 % reduces feed quality. However, for grasses NDF values less than 50 % is termed high quality feed and greater than 60 % values termed low quality feed (Van Saun, 2006). Acid detergent fiber (ADF) a subset of NDF is another measure of fiber in a feed material. This fraction contains the poorly digestible cell wall components, specifically, cellulose, lignin, and other very resistant substances. Acid detergent fibre is often used to predict energy content of feeds due to its nature. Similar to NDF, ADF is also a good measure of feed quality. Higher values within a feed suggest lower-quality feed. To maintain the quality of a feed material, the aim would be to have less than 35 % ADF in either legume or grass forages (Van Saun, 2006).

Effects of light on NDF and ADF content of forages are mixed from previous research. Lin et al. (2001) found that ADF and NDF increased in alfalfa under 50 % and 80 % artificial shade compared to 100 % light. In contrast, NDF did not differ and ADF decreased in striate lespedeza (*Kummerowia striata* Thumb.) paniculated tick trefoil, and *Desmodium canescens* L. compared to 100 % light. Johnson et al. (2002) found that NDF decreased in rhizoma peanut (*Arachis glabrata* Benth.) with increasing light.

Protein content in the form of crude protein (CP) is frequently regarded a good determinant of feed quality. Protein is estimated from the N content of feed sample. On an average basis all proteins in biological entities contain 16 % N; therefore protein content is estimated by multiplying N % by 6.25. Therefore, CP neither differentiates between N sources in feed samples (true protein or other non-protein nitrogen compounds), nor between available and unavailable protein. Despite the diversity of CP content among feeds, higher protein content is usually associated with higher feed quality in a feed. It has been observed that as forages mature, their CP is diluted with increasing fiber content. Forage fertilization practices can modify this relationship; signifying CP should not be solely used as a quality criterion without evaluating the fiber content (Van Saun, 2006).

Shade may increase N concentration, thus increasing CP concentration. Artificial shade increased CP compared to 100 % light in paniculated tick trefoil (*Desmodium paniculatum* L.) (Lin et al., 2001). For instance, there was effect of shade from 10 to11 year old radiata pine trees on CP content in alfalfa (Peri et al., 2001). However, in other instances there were no apparent effects of shade on CP. Lin et al. (2001) also found that CP content in alfalfa was not different under 50 % and 80 % artificial shade compared to 100 % light.

2.8 Summary

This review of the literature has shown the potential of BNF by legumes and actinorhizal plants in supplying N to agricultural and agroforestry systems with a potential to reduce the use of inorganic N. Accordingly, mechanisms of N transfer from the N_2-fixing plant to associated non-N_2-fixing plants were highlighted and the contribution of belowground transfer pathways reviewed. It is clear from the review that belowground N transfer mechanisms are the main and practical means of N transfer in temperate alley cropping systems as opposed to aboveground mechanism. In this thesis, I evaluated belowground N transfer pathways *en bloc* without consideration of the contribution (s) of any specific pathway (s). The review further showed that the test species considered are adapted to the climatic conditions typically occurring in Saskatchewan and have been used in some land-use systems within the province. However, biophysical interactions between the trees and associated crops in the alley cropping systems can have both positive and negative effects on the yield and nutrition of the associated crops. Minimizing negative interactions and maximizing positive ones will optimize productivity of the alley cropping systems; however, there is insufficient information on the effects of N, soil moisture and light in alley cropping systems in Saskatchewan. The overall aim of this research was to determine the impacts of the trees in alley cropping systems on the yield and nutrition of forage crops in Saskatchewan. Information generated coupled with the existing knowledge would add to the existing information on the practice of alley cropping systems within Saskatchewan, which is still at the preliminary stages of its development.

3.0 QUANTIFYING NITROGEN FIXATION OF AGROFORESTRY SHRUB SPECIES BY THE ^{15}N DILUTION TECHNIQUES UNDER GREENHOUSE CONDITIONS

3.1 Preface

The entire thesis looks at the impacts of trees in alley cropping systems on the yield and nutrition of forage crops in Saskatchewan, with emphasis on nutrients (i.e., nitrogen, N), soil moisture and light interception. With little information on the N_2-fixation capabilities of the three adapted shrub species considered in the thesis (i.e., buffaloberry, caragana and sea buckthorn), this chapter presents the results of a greenhouse experiment aimed at quantifying the N_2-fixation capabilities of the test shrub species. The results obtained may provide information on how the species can meet or complement the N requirements of forage species within Saskatchewan. This may minimize the reliance on synthetic fertilizer use, whose environmental and cost implications cannot be overemphasized. Results obtained in this chapter would be the foundation for the succeeding chapter (Chapter 4) which aims to determine how much of the fixed N_2 is transferred to associated forage crops and how that affects their yields and nutrition.

3.2 Abstract

Some land-use systems in the province of Saskatchewan include agroforestry tree/shrub species such as buffaloberry (*Shepherdia argentea* Nutt.), caragana (*Caragana arborescens* Lam.) and sea buckthorn (*Hippophae rhamnoides* L.) as important components. These species provide various ecological functions such as modifying soil moisture, light and temperature through reduction in wind speed and trapping snow, which have been widely studied. However, information on the nutrient supply function of these species, especially N_2-fixation, is limited. A greenhouse experiment was conducted at the University of Saskatchewan, Saskatoon, SK, Canada from January 2012 to April 2012. The objective of the study was to quantify N_2-fixation in buffaloberry, caragana and sea buckthorn using ^{15}N isotope-based techniques in two separate but concurrent experiments. The expectation was that the legume-*Rhizobium* (caragana) symbiosis yields higher amount of N compared to the actinorhizal-*Frankia* associations (buffaloberry and sea buckthorn). Average percentage of N derived from atmosphere (% Ndfa) in buffaloberry was 64 % in the ^{15}N dilution experiment, the average % Ndfa for caragana was 59 to 65 % and 70 to 73 % for sea buckthorn measured in the natural abundance and dilution experiments, respectively. Total amounts of N_2 fixed in the test species were also variable. Buffaloberry fixed an average of 0.25 g N tree^{-1}in the dilution experiment; the average for caragana ranged from 0.32 to 1.16 g N tree^{-1} and sea buckthorn ranged from 0.24 to 1.06 g N tree^{-1} in the natural abundance and dilution experiments, respectively within 120 DAP. This corresponds to 16 kg N ha^{-1}yr^{-1} for buffaloberry; 15 to 73 kg N ha^{-1}yr^{-1} in caragana and 11 to 67 kg N ha^{-1}yr^{-1} in sea buckthorn in the natural abundance and dilution experiments, respectively for a growing season. The amount of N_2 fixed by the species suggests that they could be used in alley cropping systems to provide sufficient N to meet the requirements of the forage and grass species grown in Saskatchewan. The tested shrub

species may therefore hold high promise to improve on-farm N management by providing N for sustaining forage crop production. This may minimize the reliance on synthetic fertilizers and thus reduce the risk of agrarian-derived soil and water pollution from excessive use of inorganic nutrient sources.

3.3 Introduction

There is a growing global concern focused on identifying and developing environmentally sustainable agroecosystems for food, fiber and fuel production that rely less on synthetic fertilizer use (Hauggaard-Nielsen et al., 2010). This means alternative sources of nutrients, especially N, need to be relied upon. Nitrogen-fixing trees in agroforestry systems hold promise in supplying the required N needed to sustain productivity of land-use systems. Trees provide additional benefits such as reducing erosion, maintaining soil fertility and providing additional income to farmers and landowners (Udawatta et al., 2002). Trees may also help in on-farm N management through mechanisms such as: 1) the "safety-net" role of their roots which take up leached nutrients, especially nitrate (NO_3^-) which has escaped the roots of crops, and pump it back into the system for use by the associated crops (Allen et al., 2004). This nutrient pumping process reduces the incidence of surface and groundwater contamination due to NO_3^- leaching and 2) biological nitrogen fixation (BNF) —a natural phenomenon in many land-use systems whereby trees/shrubs convert atmospheric N_2 to plant available N through associations with microbes (van Kessel and Hartley, 2000; Herridge et al., 2008).

Substantial amounts of N from BNF by trees or shrubs can yield positive effects for soil improvement and productivity of agroforestry and agricultural systems (Herridge et al., 2008). Herridge et al. (2008) estimated the annual inputs of N_2 fixation by pasture and fodder legumes to be 12-25 Tg and an overall input of 50-70 Tg N fixed biologically worldwide in agricultural systems. The fixed N_2 is released into the soil and subsequently taken up by associated crops through: 1) litter-fall such as in sequential systems –where fast-growing trees which accumulate large amount of foliage rich in N are rotated with crops (Gathumbi et al., 2002), and 2) root and nodule decay and root exudation, as in simultaneous systems –where trees and crops are spatially

integrated on the same piece of land (Khanna, 1998; Peoples and Herridge, 1990). However, the amount of N input through BNF is subject to factors such as soil, climate, tree-microbe interactions and management conditions (Mafongoya et al., 2004).

Despite the BNF role played by N_2-fixing trees and shrubs in the functioning of many ecosystems, it is difficult to quantify the amounts of their N_2 fixed due to: 1) diversity in occurrence, and large plant-to-plant variation in growth and nodulation status of N_2-fixing species 2) long-term, perennial nature of growth and the seasonal or year-to-year changes in patterns of N assimilation; and 3) logistical limitations of working with mature trees which are generally impractical to harvest in their entirety (Boddey et al., 2000). These limitations make only few of the available techniques for estimating N_2-fixation applicable to deep-rooted trees and shrubs (Gathumbi et al., 2002). The technique that holds the most promise for quantifying contributions of BNF in trees is the ^{15}N isotope-based technique (i.e.,^{15}N natural abundance and isotope dilution) (Boddey et al., 2000).

The ^{15}N isotope dilution technique which involves growing plants in ^{15}N-enriched soil or other growing medium, as opposed to exploiting the naturally-occurring soil ^{15}N enrichment (natural abundance), is appropriate for annual crops and herbaceous N_2-fixing plants. The advantages of the ^{15}N isotope dilution method are that high analytical sensitivity is not required and that the potential influence of isotope fractionation processes in the soil and in the N_2 fixation process is reduced (Chalk, 1991; Carranca et al., 1999). However, it has some limitations including, disturbance of the agroecosystem due to the application of ^{15}N-enriched fertilizer in established agroecosystems; high costs of the ^{15}N enriched fertilizer, making the technique applicable to a limited area of land, and low accuracy in quantifying low levels of symbiotic N_2-fixation (Hardarson et al., 1988).

The natural abundance technique exploits naturally occurring differences in ^{15}N composition between plant-available N sources in the soil and that of atmospheric N_2 (Boddey et al., 2000). It is based on the assumption that if N_2-fixing plants derive and incorporate atmospheric N_2, the plant $\delta\ ^{15}N$ would be closer to the atmospheric standard $\delta\ ^{15}N$ (0.3663 ‰) as compared to non-N_2-fixing species using other sources of plant available N such as soil and fertilizer (Miller, 2011). The principal assumption of the natural abundance technique is that the ^{15}N enrichment of the reference plants accurately reflects the ^{15}N enrichment of the soil N taken up by fixing plants. A significant difference in $\delta\ ^{15}N$ between reference plants and supposed N_2-fixing plants is required to show active N_2-fixation (Shearer and Kohl, 1993). The ^{15}N natural abundance technique has several advantages over the isotope dilution technique; it does not require ^{15}N fertilizer application hence there is no problem with non-uniform labeling of trees or shrubs within the rooting zone and there is no disturbance to established agroecosystems. The natural abundance protocol, despite requiring very high analytical sensitivity, is therefore, the most reliable among the ^{15}N isotope-based methods for trees (Eriksen and Høgh-Jensen, 1998).

In this study both the ^{15}N natural abundance and the isotope dilution techniques were employed in separate but simultaneous experiments in order to curtail the potential problem of undetectable $\delta\ ^{15}N$ natural abundance in the soil used. The ultimate goal was to provide reliable estimates of BNF in the test species, to identify their potential contribution to N management in land-use systems. The objective was to quantify BNF by ^{15}N natural abundance and isotope dilution techniques. Buffaloberry and sea buckthorn are actinorhizal plants of the family *Elaeagnaceae.* Due to their ability to fix atmospheric N_2 in a symbiotic association with *Frankia*, they thrive well on impoverished soils (Gentili and Huss-Danell, 2002; Miller, 2011). Caragana belongs to the family *Leguminosae* and fixes atmospheric N_2 in association with *Rhizobium*,

hence thrives on low N fertility soils (Pollock and Svendsen, 2008). Because symbiotic systems with leguminous plants and rhizobia have the greatest quantitative impact on the N cycle (Predeepa and Ravindran, 2010), it was hypothesized that the legume-*Rhizobium* (caragana) symbiosis would yield higher amount of N compared to the actinorhizal-*Frankia* associations (buffaloberry and sea buckthorn).

3.4 Materials and Methods

3.4.1 Experimental design and treatments

A greenhouse experiment was conducted at the University of Saskatchewan, Saskatoon, SK, Canada from January 2012 to April 2012 to quantify N_2-fixation using the ^{15}N dilution techniques. Treatments were three N_2-fixing tree species; buffaloberry (*Shepherdia argentea* Nutt.), caragana (*Caragana arborescens* Lam. "Ross") and sea buckthorn (*Hippophae rhamnoides* L. "Indian summer"). Choke cherry (*Prunus virginiana* var *melanocarpa* L.) was used as the non-N_2-fixing reference plant for calculations of % Ndfa. Separate but simultaneous experiments were established for each method of the ^{15}N dilution techniques (^{15}N natural abundance and ^{15}N isotope dilution). Within each experiment, pots were arranged in a completely randomized design (CRD) with six replicates for each species, giving a total of 48 pots for both experiments (24 pots for each experiment). Six replicates of three N_2-fixing species with reference species. The greenhouse had the following average conditions throughout the experimental period: light intensity varied from 167 to 411 μmol m^{-2} s^{-1}, mean day/night temperatures were 23/21 °C, mean day/night relative humidity 31/38 % and 18/6 h day/night rhythm.

3.4.2 Growth medium

The growth medium was Brown Chernozemic soil from an agricultural field located in Central Butte ca. 200 km south of Saskatoon, SK. Crop rotation on the site until 1990 was wheat-fallow and wheat-pea-canola since 1990. The most recent crop grown on the site prior to soil collection was wheat. The soil fertility management had been annual application of urea fertilizer at a rate of 40 to 50 kg N ha^{-1} for canola and wheat. For all crops, application of monoammonium phosphate fertilizer was at a rate of 15 to 20 kg P_2O_5 ha^{-1}. The pea crop always received commercial *Rhizobium* inoculant. The soil, with a bulk density of 1.10 g cm^{-3} was mixed with quartz sand in a ratio of 9:1 (i.e., ca. 12.0 kg soil and 1.2 kg sand) per pot. To ensure that the appropriate microorganisms necessary for BNF were in the treatment soils, soil ′inoculant′ was collected from the rooting zone of the test tree species in the field. Mature, healthy stands of the test trees were identified at the experimental site of Agroforestry Development Centre's research farm, Indian Head, SK and soil close to the roots, collected. The ′inoculant′ soil was collected in the top 0 to 60 cm of the soil profile at 0.50 m radius around the trees using a shovel and a hand trowel. A total soil weight of 30 kg was collected and returned to the laboratory. The soil ′inoculant′ was stored at -4 ^{o}C until used. A subsample (0.50 kg) of the ′inoculant′ soil was placed in a band around the rooting zone of the seedlings in the pots. Bare-rooted seedlings from the Agroforestry Development Centre –Indian Head, were cold-hardened by storing at -4 ^{o}C in a freezer for 90 days. Plants were grown for 120 days from bare-rooted seedlings after thawing at room temperature for 48 hours. Two seedlings were planted per pot and 30 days after planting (DAP) they were thinned to one per pot. The plants for the ^{15}N isotope dilution experiment were labeled with aqueous ^{15}N-enriched $NH_4^+NO_3^-$ solution (10 atom % excess) at a rate of 5 kg ha^{-1}. The solution was applied 30 days after the sprouting of seedling to allow for proper root

establishment. This was done by dissolving the ^{15}N-enriched $NH_4^+NO_3^-$ in 1.2 L of distilled water, 50 mL of solution was applied to each pot around the root of the test species with the aid of a syringe. The plants were watered every two days with tap water with a volume of water required to bring soil to ca. 80 % field capacity throughout the experimental period.

3.4.3 Soil sampling and analysis

Initial analysis of the soil was done (Table 3.1) for total inorganic N (NO_3^- and NH_4^+) after extraction with 2*M* KCl solution (Maynard et al., 2008) and available phosphorus (P) by using the modified Kelowna method (Qian et al., 1994). These analyses were done on the extracts colorimetrically using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA). Exchangeable Ca^{2+}, K^+ and Mg^{2+} were analyzed following extraction with 1*M* ammonium acetate solution using an atomic absorption spectrometer (Varian SpectrAA 220, Burladingen, Germany) (Hendershot et al., 2008). Available sulphur (S) was determined on an autoanalyzer following 0.01*M* $CaCl_2$ extraction (Hu et al., 2005). Organic carbon (OC) was determined by dry combustion using a LECO C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA), pH and electrical conductivity (EC) (1:2 soil:water, Hendershot et al., 2008) were determined using a Beckman 50 pH Meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA), respectively. The $\delta^{15}N$ of the soil N pool was determined on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany).

Table 3.1. Physicochemical properties of Brown Chernozemic soil from a wheat field, used in the greenhouse experiment to quantify N_2-fixation in three shrubs.

Parameter	Value
pH (1:2 soil:water)	8.02†
Electrical conductivity (1:2 soil: water) (mS cm^{-1})	0.11
Available phosphorus (mg kg^{-1})	80.0
Available inorganic N (NO_3^-) (mg kg^{-1})	9.40
Available inorganic N (NH_4^+) (mg kg^{-1})	2.10
Available sulphur (S) (mg kg^{-1})	4.88
Organic carbon (OC) (g kg^{-1})	11.75
Exchangeable K^+ (cmol (+) kg^{-1})	0.53
Exchangeable Mg^{2+} (cmol (+) kg^{-1})	2.30
Exchangeable Ca^{2+} (cmol (+) kg^{-1})	3.86
$\delta^{15}N$ of soil N pool (‰)	7.94

†Mean values (n = 4)

3.4.4 Plant sampling and analysis

Only one replicate out of six of buffaloberry in the ^{15}N natural abundance had one nodule. For this reason, that was the only replicate used in all the calculations for buffaloberry. Also, due to poor growth of some trees in the natural abundance experiment, only four replicates of each of caragana and sea buckthorn were used. Only visibly healthy plants were included in the analyses. In the ^{15}N dilution experiment, all of the six replicates were visibly healthy and included in the analysis.

One hundred and twenty days after planting (DAP), the shrubs were harvested and partitioned into shoots, roots and nodules for N_2-fixing plants and shoots and roots for the non-N_2-fixing plant. The nodules were removed from the roots and counted manually and oven-dried at

60 °C. The shoot and root materials were oven-dried at 60 °C to stable weight to determine dry biomass, ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ), and re-ground to fine powder using a ball grinder (8000D Mixer/Mill, SPEX SamplePrep® LLC., Metuchen, NJ, USA). The ground plant samples were weighed using a micro balance (Sartorius Microbalance, CPA2P, Bradford, MA, USA) and subsamples encapsulated in 8 mm × 5 mm tin capsules.

3.4.4.1 ^{15}N natural abundance samples

Samples (3.0 mg ± 0.3 mg) were analyzed for δ^{15}N and N concentration on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany). The standard deviation of repeated measurements (n = 12) of laboratory standards was ± 0.15 ‰. The percentage of N derived from the atmosphere (% Ndfa) was assessed as follows (Ledgard, 1989):

$$\%Ndfa = \left(\frac{\delta^{15}N_{non-fixingtree} - \delta^{15}N_{fixingtree}}{\delta^{15}N_{non-fixingtree} - B} \right) \times 100\% \qquad [3.1]$$

where B is the δ^{15}N of the N_2-fixing plant growing without any added N (i.e., relying solely on BNF) (Unkovich et al., 1994). This is accomplished by growing plants in N-free media, usually in hydroponics. Typical range of B values in woody N_2-fixing plants is -2.0 to +1.0 ‰ (Boddey et al., 2000). The B values for the test species were assumed to be 0 ‰ (Busse et al., 2007). The δ^{15}N for the samples were expressed in units of per mil or parts per thousand (‰) with respect to the atmospheric N_2 as the standard Shearer and Kohl, (1993):

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{std}} - 1 \right) \times 1000‰ \qquad [3.2]$$

where R is the ratio of ^{15}N/^{14}N of either the sample or standard.

The amount of the N_2 fixed was determined from: 1) the relative reliance of the crop upon N_2 fixation for growth [i.e., the percentage of the plant N derived from atmospheric N_2, (% Ndfa)] and, 2) the amount of N accumulated in the N_2-fixing plant as detailed by Peoples et al. (2009):

$$N_{fixed} = \frac{\%Ndfa}{100} \times N_{fixingtree} \quad [3.3]$$

$$N_{fixingtree} = \frac{\%N}{100} \times DryMatter_{Nfixingtree} \quad [3.4]$$

3.4.4.2 ^{15}N isotope dilution samples

Samples (2.0 mg ± 0.2 mg) were analyzed for atom % ^{15}N excess and total N concentration with same instrumental procedure as in section 3.4.4.1. The % Ndfa was calculated according to the method by Rennie and Dubetz (1986):

$$\%Ndfa = 1 - \frac{(atom\%^{15}Nexcess_{fixingtree})}{(atom\%^{15}Nexcess_{nonfixingtree})} \times 100\% \quad [3.5]$$

The amount of the N_2 fixed in both experiments was determined using equations [3.3 & 3.4]. To account for the total N_2-fixation in the whole plant, the equation used by Robinson et al. (2000) was used to calculate the δ^{15}N and ^{15}N in the whole plant as an average of shoot and root δ^{15}N and^{15}N weighted by the total N contents (mg) of shoots and roots as detailed below:

$$\delta^{15}N_{wholeplant} = \frac{\sum(\delta^{15}N_{root} \times N_{root} + \delta^{15}N_{shoot} \times N_{shoot})}{\sum(N_{root} + N_{shoot})} \quad [3.6]$$

3.4.5 Statistical analysis

Except nodule number and dry weight which were natural log (ln) transformed to stabilize the variance and fulfill the assumption of normality, all parameters were normally distributed

based on the Shapiro-Wilk test (SAS, 2008). Total amount of N_2-fixed, % Ndfa in roots, shoots and whole plant as well as plant biomass, N content in root and shoot, and nodule productivity in all test species was analyzed using SAS 9.2 for Windows (SAS, 2008) using one-way analysis of variance (ANOVA) by the PROC GLM procedure at 5 % level of significance. Following ANOVA, Tukey's Honestly Significant Difference (HSD) was used to separate treatment means when significant. The SAS macro pdmix800 was used to perform pairwise comparisons at 5 % level of significance (Saxton, 1998). Correlation analysis was performed using PROC CORR procedure in SAS with whole plant and shoot % Ndfa, and whole plant and shoot N_2-fixed as parameters of interest correlated with nodule productivity and plant biomass.

3.5 Results

3.5.1 Shoot and root dry matter yield

There were large differences in plant biomass yield between the two experiments. The isotope dilution experiment had about 2 to 4 fold higher shoot dry matter and root dry matter in all the test species compared to the values in the ^{15}N natural abundance experiment (Fig. 3.1A and 3.1B). Caragana had the largest shoot ($P = 0.0048$) and root ($P < .0001$) dry matter yields in the ^{15}N natural abundance experiment. In the ^{15}N isotope dilution experiment, caragana had the largest root dry matter yield ($P < .0001$), while sea buckthorn had the largest shoot dry matter yield ($P < .0001$) (Fig. 3.1A and 3.1B). Lowest plant biomass values were measured in buffaloberry in both experiments.

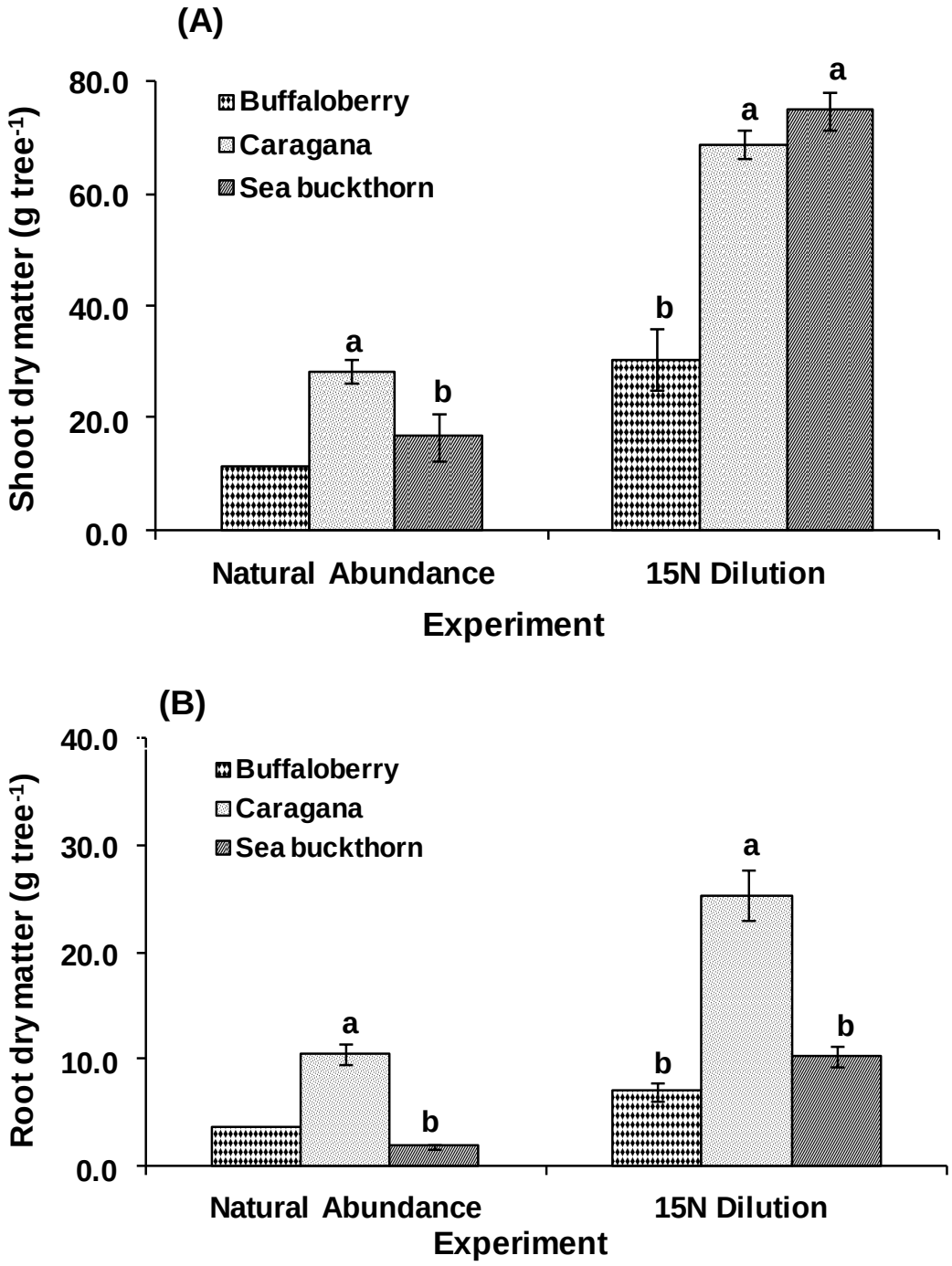

Fig. 3.1. Shoot (A) and root (B) dry matter (g tree^{-1}) measured in the ^{15}N natural abundance (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) and ^{15}N isotope dilution experiments (n = 6). Vertical bars show standard error of the mean. Means followed by same letters within an experiment are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

3.5.2 Nodule productivity

There were significant differences ($P \leq 0.05$) in nodule numbers and nodule dry weight among the test species. Nodulation was higher in caragana than in sea buckthorn and buffaloberry in both the ^{15}N natural abundance ($P = 0.0015$) and ^{15}N isotope dilution ($P < .0001$) experiments (Fig. 3.2A). Comparatively, the number of nodules in caragana in the ^{15}N isotope dilution experiment was 2.5 times higher than the number obtained in the ^{15}N natural abundance experiment (Fig. 3.2A). However, nodule dry matter was highest in sea buckthorn in both the ^{15}N natural abundance ($P < .0001$) and ^{15}N isotope dilution ($P = 0.0002$) experiments, though it produced fewer nodules than caragana, reflecting the large size of nodules in the sea buckthorn. Buffaloberry produced the lowest nodule numbers and dry weight (Fig. 3.2B).

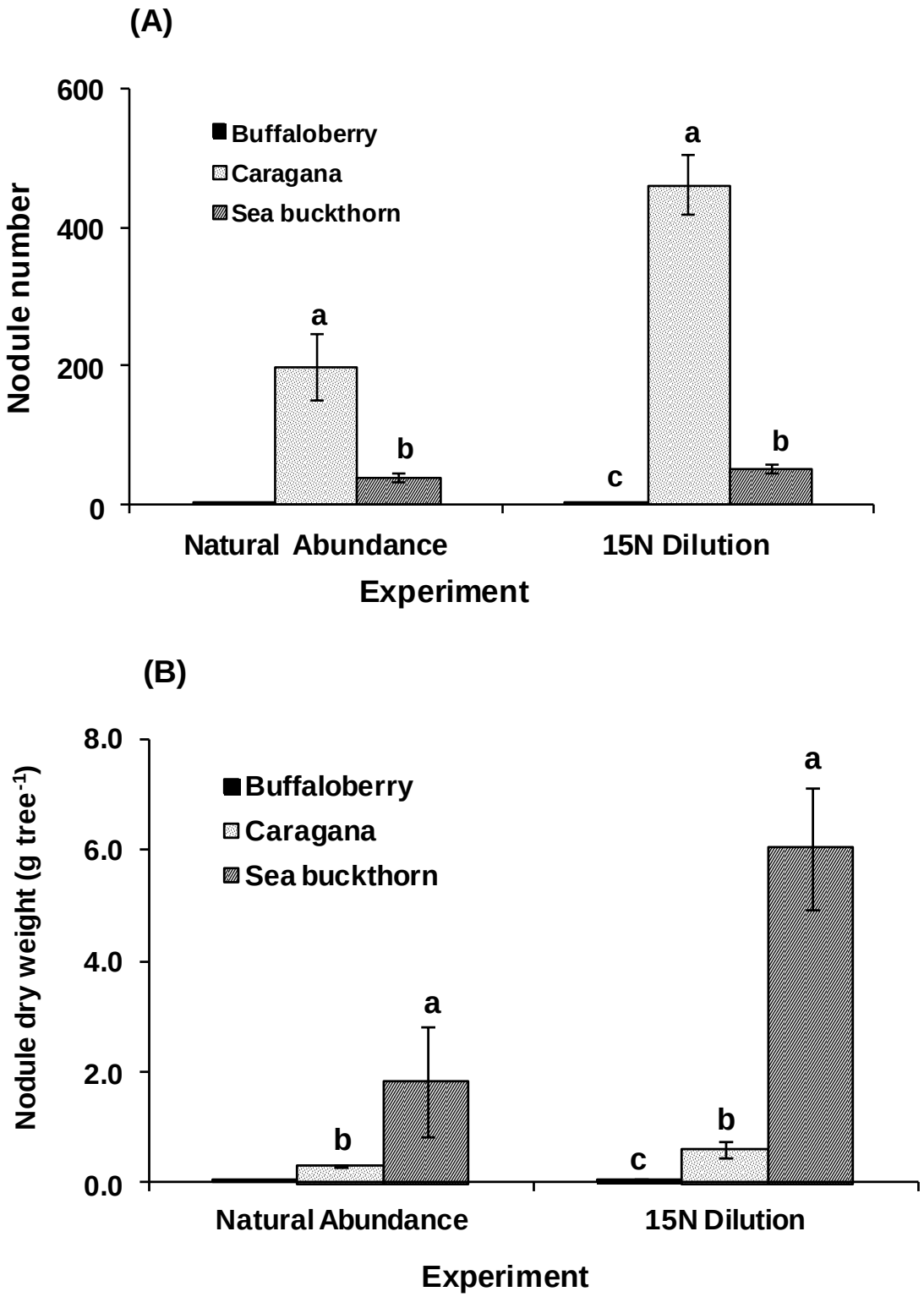

Fig. 3.2. Nodule number (A) and nodule dry weight (B) measured in the ^{15}N natural abundance (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) and ^{15}N isotope dilution (n = 6) experiments. Vertical bars show standard error of the mean. Means followed by same letters within an experiment are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

3.5.3 Percentage of nitrogen derived from the atmosphere

There were significant differences in % Ndfa in roots ($P = 0.0002$), shoots ($P = 0.0007$) and whole plants ($P = 0.0506$) in the ^{15}N natural abundance experiment (Fig. 3.3A). Roots of sea buckthorn had about thrice as much % Ndfa (77 %) as roots of caragana and buffaloberry (< 30 %). Both caragana and sea buckthorn had similar and higher levels of % Ndfa in shoots and whole plant than buffaloberry (Fig. 3.3A) based on $\delta^{15}N$ and ^{15}N values (Appendix C). In contrast, in the ^{15}N isotope dilution experiment, % Ndfa in all the plant components was not significantly different among the test species ($P > 0.05$). Sea buckthorn had a higher root, shoot and whole plant % Ndfa followed by caragana and then buffaloberry. Apart from % Ndfa in buffaloberry, the % Ndfa in all plant components was similar in both experiments for caragana and sea buckthorn (Fig. 3.3). Buffaloberry had lower % Ndfa (3-14 %) in the ^{15}N natural abundance experiment compared to the ^{15}N isotope dilution experiment (41-64 %).

3.5.4 Amounts of nitrogen fixed in test species

In contrast to the trend observed for % Ndfa, the amount of N_2 fixed was higher in caragana in all of the plant components compared to sea buckthorn in the ^{15}N natural abundance experiment (Fig. 3.4A). On a whole plant basis, caragana fixed 0.32 g N tree^{-1} and sea buckthorn fixed 0.24 g N tree^{-1}. In the ^{15}N isotope dilution experiment, caragana fixed very similar amounts of N (1.16 g N tree^{-1}) compared to sea buckthorn (1.06 g N tree^{-1}). Buffaloberry fixed less than one quarter the amount of N than the other two species (0.25 g N tree^{-1}) (Fig. 3.4B). The amount of N_2 fixed in shoot and whole plant in all the test species were higher in the ^{15}N isotope dilution (3 to 4 times) than corresponding values in the ^{15}N natural abundance experiments. However, the reverse trend holds in the roots of buffaloberry.

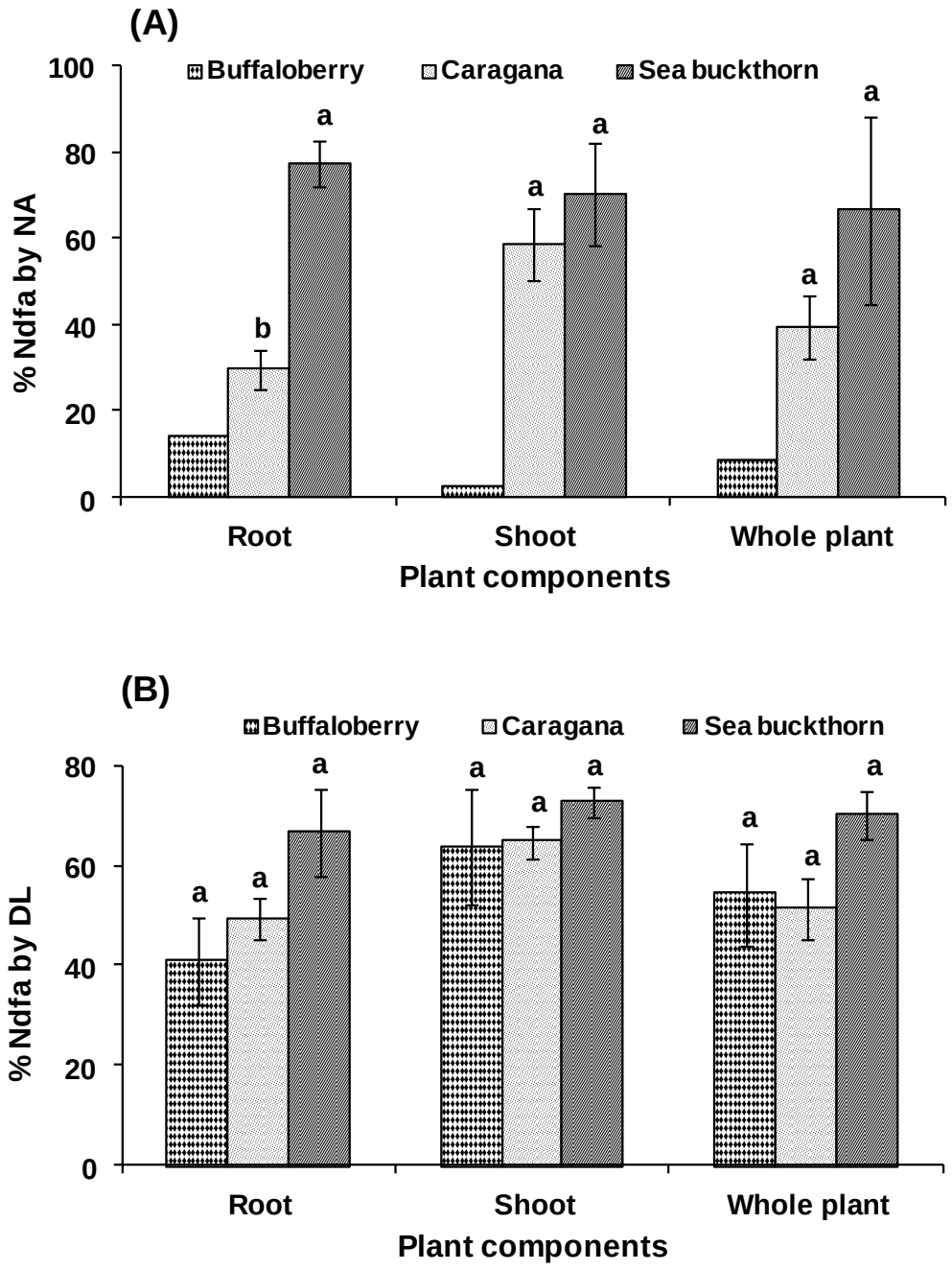

Fig. 3.3. Percentage of nitrogen derived from the atmosphere (% Ndfa) among plant components quantified in the ^{15}N natural abundance, NA (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) (A) and ^{15}N isotope dilution, DL (n = 6) (B) experiments. Vertical bars show standard error of the mean. Means followed by same letters within a plant component are not significantly different at $P \leq 0.05$ according to Tukey`s HSD.

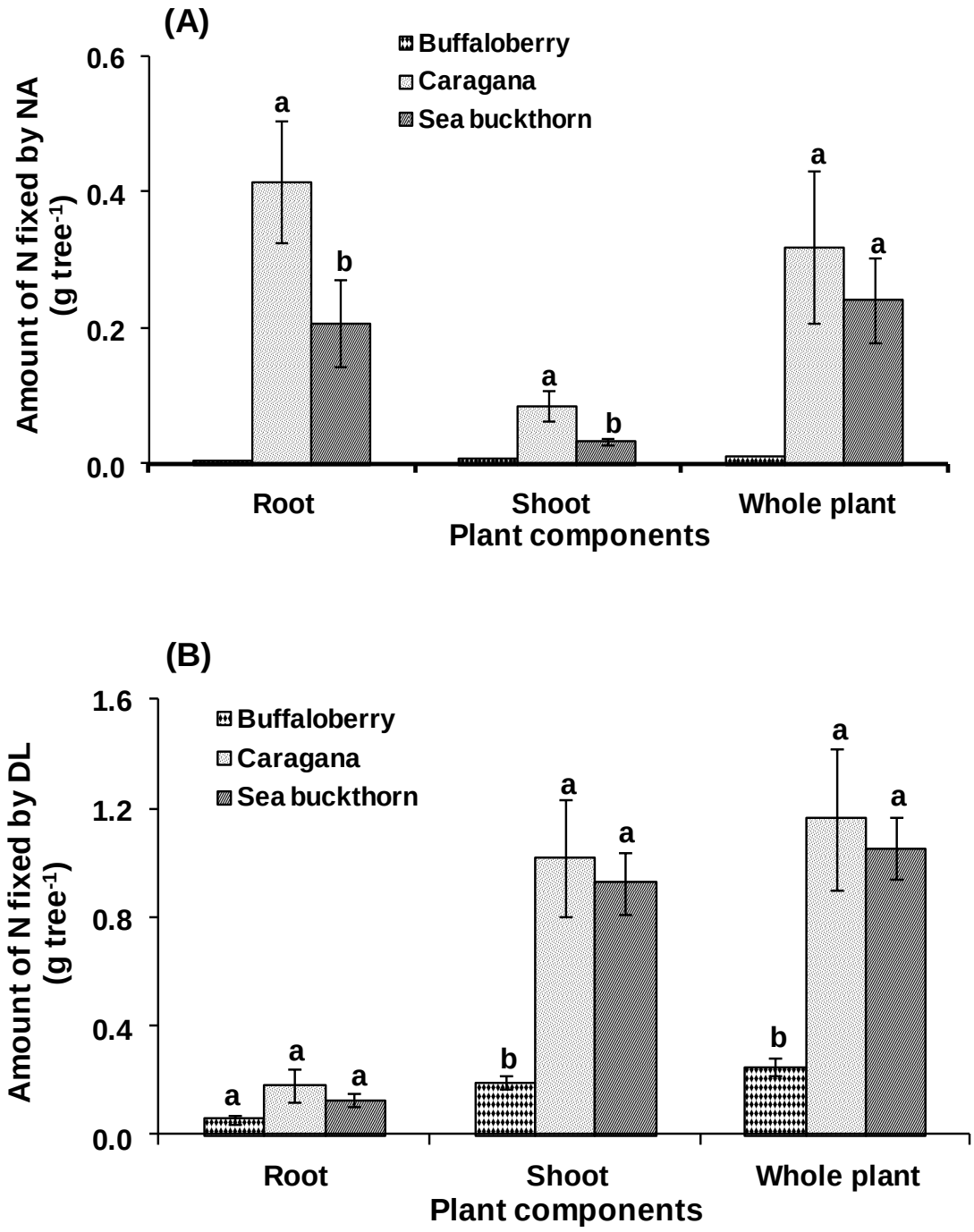

Fig. 3.4. Amount of N_2 fixed among plant components measured in the ^{15}N natural abundance, NA (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) (A) and ^{15}N isotope dilution, DL (n = 6) (B) experiments. Vertical bars show standard error of the mean. Means followed by same letters within a plant component are not significantly different at $P \leq 0.05$ according to Tukey`s HSD.

3.5.5 Shoot and root percentage nitrogen

Caragana had the highest shoot % N (3 %). The lowest % N values (1.5 %) were measured in buffaloberry in both the ^{15}N natural abundance (P = 0.0027) and ^{15}N isotope dilution (P = 0.0004) experiments (Fig. 3.5A). There were significant differences among the species in root % N in the ^{15}N natural abundance experiment (P = 0.0477) but not in the ^{15}N isotope dilution experiment (P = 0.6139). Buffaloberry had the highest root % N; the lowest mean values in root % N in both experiments were measured in sea buckthorn (Fig. 3.5B).

3.5.6 Shoot and root nitrogen content

Caragana had the highest value of shoot N content in both the ^{15}N natural abundance (P = 0.015) and ^{15}N isotope dilution (P = 0.0003) experiments. The lowest shoot N content value was measured in buffaloberry (Fig. 3.6A). The trend was generally the same in root N content as in shoot N content with caragana having the highest (P = 0.0002) (Fig. 3.6B). Generally, caragana which had the highest shoot and root N accumulation in both experiments had the highest shoot and root dry matter yield, while buffaloberry with the least N accumulation in shoot had the lowest shoot dry matter yield (Figs.3.1A & B and 3.6A).

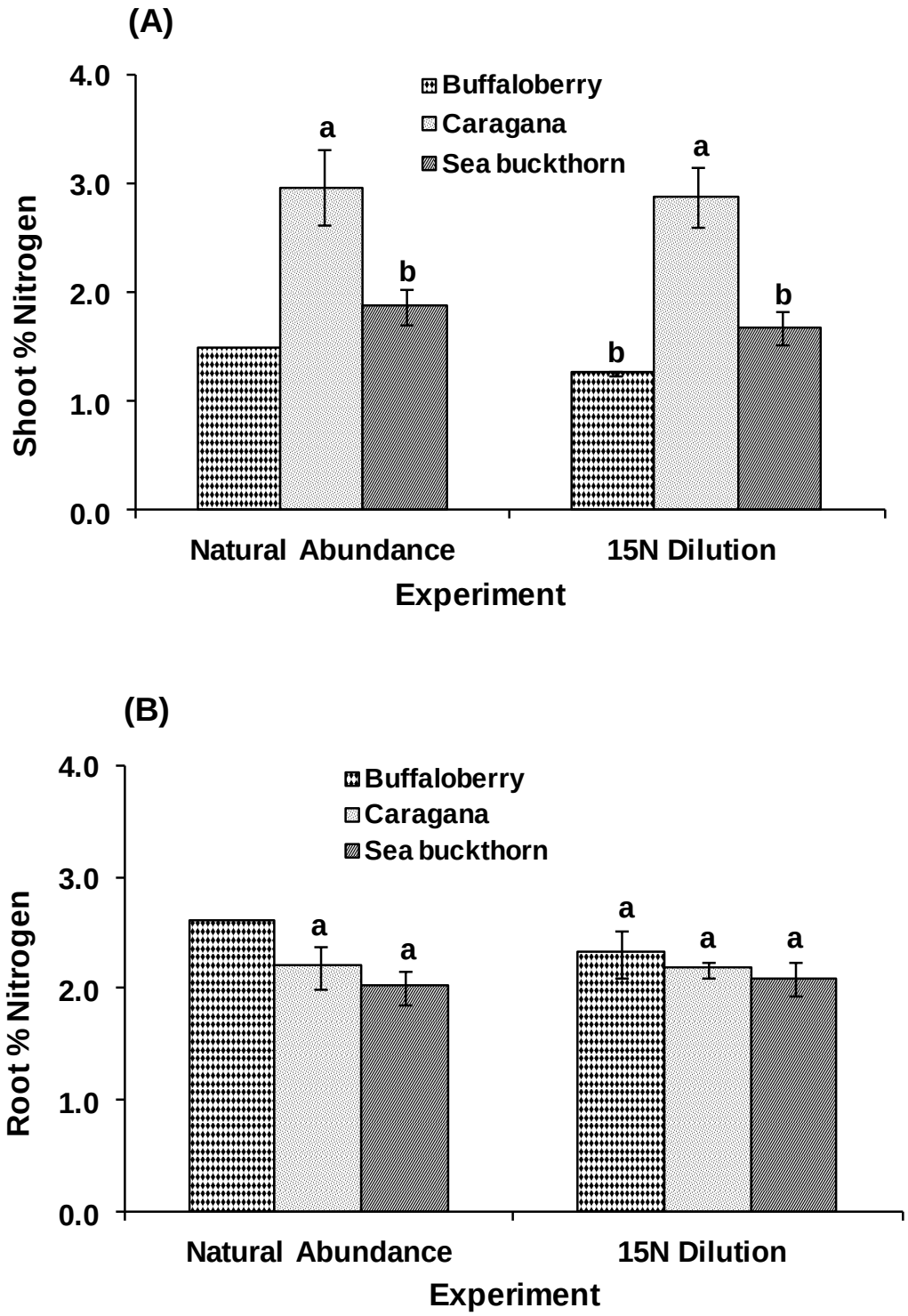

Fig. 3.5. Shoot (A) and root (B) % N measured in the ^{15}N natural abundance (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) and ^{15}N isotope dilution (n = 6) experiments. Vertical bars show standard error of the mean. Means followed by same letters within an experiment are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

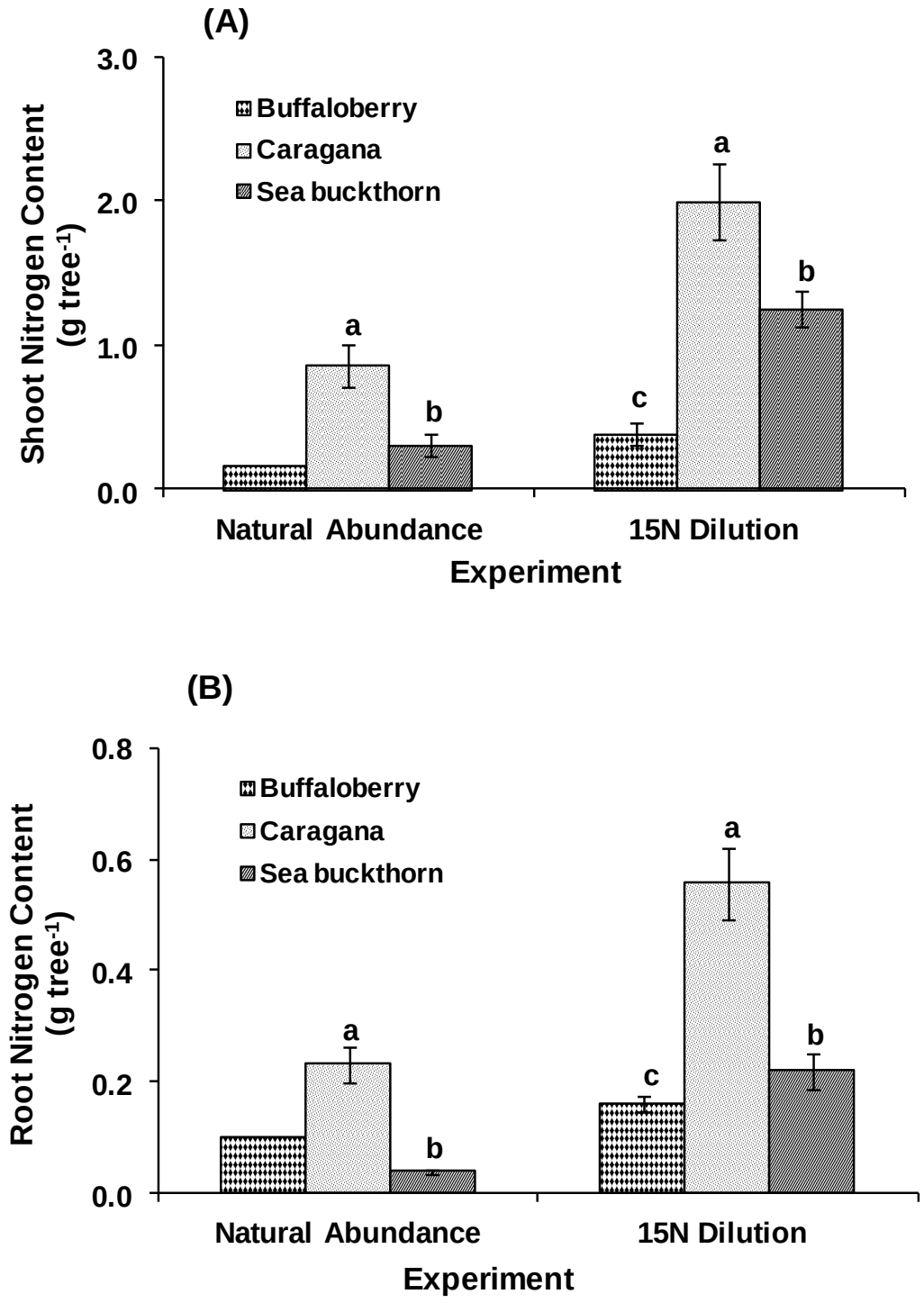

Fig. 3.6. Shoot (A) and root (B) N content measured in the ^{15}N natural abundance (n = 4 for caragana and sea buckthorn, n = 1 for buffaloberry) and ^{15}N isotope dilution (n = 6) experiments. Vertical bars show standard error of the mean. Means followed by same letters within an experiment are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

3.5.7 Correlation analysis

Correlations between shoot dry matter and N_2-fixed in the whole plant was significant in ^{15}N natural abundance (r = 0.84, *P* <.0001) and ^{15}N isotope dilution (r = 0.87, *P* = 0.0002) experiments (Tables 3.2 and 3.3). Similarly, shoot dry matter and N_2-fixed in shoot were significantly correlated in the ^{15}N natural abundance (r = 0.86, *P* <.0001) and ^{15}N isotope dilution (r = 0.63, *P* = 0.0272) experiments. In the ^{15}N natural abundance and ^{15}N isotope dilution experiments, nodule dry weight was correlated with whole plant % Ndfa (r = 0.59, *P* = 0.0445) (Table 3.2) and shoot % Ndfa (r = 0.64, *P* = 0.0259) (Table 3.3), respectively. Finally, nodule number was correlated with whole plant N_2-fixed in the ^{15}N isotope dilution experiment (r = 0.92, *P* < .0001) (Table 3.3).

Table 3.2. Correlations between measured percentages of nitrogen derived from the atmosphere (% Ndfa) and N_2-fixed with nodule productivity and plant biomass in the ^{15}N natural abundance experiment in caragana under greenhouse conditions.

	WPNdfa †	WPNfix ‡	SNdfa §	SNfix ¶	SDM #	RDM ††	NN ‡‡
WPNfix	0.309 §§	1.000					
	0.213 ¶¶						
SNdfa	0.894	0.191	1.000				
	<.0001***	0.448					
SNfix	0.250	0.992	0.159	1.000			
	0.318	<.0001***	0.529				
SDM	0.087	0.844	-0.087	0.864	1.000		
	0.7300	<.0001***	0.7307	<.0001***			
RDM	-0.245	0.540	-0.125	0.544	0.439	1.000	
	0.327	0.021*	0.622	0.020*	0.068		
NN	-0.194	0.458	0.051	0.491	0.335	0.938	1.000
	0.546	0.135	0.875	0.105	0.298	<.0001***	
NDW ##	0.588	0.362	0.479	0.362	0.575	-0.183	-0.280
	0.045*	0.247	0.115	0.248	0.051	0.569	0.378

† WPNdfa, whole plant %Ndfa
‡ WPNfix, whole plant amount of N_2 fixed
§ SNdfa, shoot %Ndfa
¶ SNfixed, shoot amount of N_2 fixed
# SDM, shoot dry matter
†† RDM, root dry matter
‡‡ NN, nodule number
§§ Coefficient of correlation, r
¶¶ *P* values
NDW, nodule dry weight
* = significant at 5%
** = significant at 1%
*** = significant at 0.1%
n = 8 and $P \leq 0.05$

Table 3.3. Correlations between measured percentages of nitrogen derived from the atmosphere (% Ndfa) and N_2-fixed with nodule productivity and plant biomass in the ^{15}N isotope dilution experiment in caragana under greenhouse conditions.

	WPNdfa†	WPNfix ‡	SNdfa §	SNfix ¶	SDM #	RDM ††	NN ‡‡
WPNfix	0.163 §§ 0.757 ¶¶	1.000					
SNdfa	0.588 0.2201	0.584 0.046*	1.000				
SNfix	-0.441 0.382	0.668 0.018*	0.335 0.287	1.000			
SDM	0.107 0.840	0.871 0.0002**	0.368 0.239	0.63269 0.0272*	1.000		
RDM	-0.614 0.194	0.615 0.033*	0.108 0.739	0.79247 0.0021*	0.644 0.024*	1.000	
NN	-0.312 0.547	0.916 <.0001***	0.384 0.219	0.57507 0.0504*	0.849 0.0005**	0.706 0.010*	1.000
NDW ##	0.652 0.161	0.246 0.440	0.637 0.026*	-0.0979 0.7623	-0.031 0.923	-0.337 0.284	-0.080 0.805

† WPNdfa, whole plant %Ndfa
‡ WPNfix, whole plant amount of N_2 fixed
§ SNdfa, shoot %Ndfa
¶ SNfixed, shoot amount of N_2 fixed
# SDM, shoot dry matter
†† RDM, root dry matter
‡‡ NN, nodule number
§§ Coefficient of correlation, r
¶¶ *P* values
NDW, nodule dry weight
* = significant at 5%
** = significant at 1%
*** = significant at 0.1%
n = 18 and $P \leq 0.05$

3.6 Discussion

3.6.1 Shoot and root dry matter

The higher biomass yields, % Ndfa and total N_2 fixed by plants in the ^{15}N isotope dilution experiment relative to the ^{15}N natural abundance experiment suggests that the symbiotic relationship in the test species was not adversely affected by the applied ^{15}N enriched fertilizer. Despite the ^{15}N enriched fertilizer dose being relatively low (5 kg ha^{-1}) and intended for tracing purposes rather than testing the nutritional effects, it seemed to have influenced growth of the test species. The test species produced higher amount of biomass (2 to 4 folds) without affecting their BNF capabilities. The ^{15}N-enriched fertilizer may have induced early growth leading to higher and enhanced nodulation and higher BNF (Voisin et al., 2002), leading to higher biomass yield and total N_2 fixed.

The fertilizer effect might have also led to the efficient use of available resources as addition of N fertilization in forage species increases yield and reduces the amount of dead material, which aids the plants to effectively utilize light, nutrients and water (Zemenchik and Albrecht, 2002). The higher plant biomass in caragana compared to buffaloberry and sea buckthorn may be because caragana produces higher amount of biomass within a short growing period (Marchand and Masse, 2008). This higher biomass production may have increased the amount of N_2 fixed due to higher demand for N for growth (Busse, 2000).

3.6.2 Nitrogen fixation capacity of tree/shrub species

Estimates of % Ndfa were similar between the ^{15}N isotope dilution and natural abundance experiments, especially for caragana and sea buckthorn. However, % Ndfa estimates for buffaloberry were lower in the ^{15}N natural abundance experiment compared to the ^{15}N isotope dilution experiment. In the ^{15}N natural abundance experiment, only one plant was nodulated

compared to four in caragana and sea buckthorn. In both experiments, only those plants that were nodulated were used to calculate % Ndfa. The observed higher shoot and whole plant % Ndfa in sea buckthorn relative to buffaloberry and caragana could be attributed to differences in nodule dry matter as there was a significant positive correlation between whole plant shoot % Ndfa and nodule dry weight (r = 0.59, *P* = 0.0445) in the natural abundance experiment. There was also a significant positive correlation between shoot % Ndfa and nodule dry weight (r = 0.64, *P* = 0.0259) in the ^{15}N isotope dilution experiment. The results corroborate those of del Rosario et al. (1997) and Hayat et al. (2008) who reported that nodule dry weight correlated with % Ndfa in mungbean. Similarly, Kyei-Boahen et al. (2001; 2002) reported a linear correlation (*r* = 0.82) between nodule dry weight and % Ndfa in chickpea. Furthermore higher % Ndfa in sea buckthorn might have been due to the consistently greater average values of % Ndfa for actinorhizal plants than for legumes across studies (Andrews et al., 2011).

The variations in % Ndfa among roots, shoots and whole plant components of a single plant may be due to the markedly different $\delta^{15}N$ signature variation between the different parts. These differences may be due to NH_4^+ and NO_3^- acquisition, preferential NO_3^- reduction in roots and shoots, and N_2 fixation potential (Shearer et al., 1980; Robinson et al., 2000; Unkovich et al., 2000; Evans, 2001). Fixation values obtained in this study compares with work by Parrota et al. (1994) and Binkley (1997) who recorded % Ndfa values from 48 to 100 % for a variety of temperate zone and tropical tree species using the ^{15}N dilution method.

When the ^{15}N isotope dilution technique is used, the choice of reference plants often affects estimation of BNF due to low ^{15}N variability in the rooting profile (Boddey et al., 2000; Yang et al., 2011). The slight variation in the % Ndfa values of the same species by the two techniques could not have been due to reference species and uniformity in ^{15}N, because the reference species

used is same and of the same age and uniformity of ^{15}N of the soil was ensured by physically mixing the composited soil before the experiment (Boddey and Victoria, 1986). In such "homogeneous" soils, plants have access to similar ^{15}N abundance regardless of any differences in rooting depth or architecture between N_2-fixing and non-N_2-fixing plants (Boddey et al., 2000).

3.6.3 Contribution of tree/shrub species to nitrogen management

Nitrogen fertilization in forage species increases yield and decreases dead material. It also reduces the concentration of neutral detergent fiber while increasing crude protein, as well as increasing the competitive ability of the forages in utilizing light, nutrients, and water (Zemenchik and Albrecht, 2002).

Amounts of N_2 fixed by the three tree species varied depending on the experiment. Because the estimate of the amount of N_2 fixed is related to biomass, the smaller initial trees grown in the ^{15}N natural abundance experiment necessarily reflect lower amounts of N_2 fixed. Fixed N_2 in the ^{15}N natural abundance experiment reflects the 120 day growth period of the trees, whereas fixed N_2 in the ^{15}N dilution experiment reflects the 90 day period in which the trees were accessing the ^{15}N-NH_4NO_3. Assuming a growing period of approximately 160 days, the amount of N_2 fixed ranged from 15 kg N $ha^{-1}yr^{-1}$ to 73 kg N $ha^{-1}yr^{-1}$ for caragana, 11 kg N $ha^{-1}yr^{-1}$ to 67 kg N ha^{-1} yr^{-1} for sea buckthorn and 16 kg N $ha^{-1}yr^{-1}$ for buffaloberry (as shown in appendix D). The estimated annual amount of N_2 fixed by caragana is in the range of reported values by Gathumbi et al. (2002) who reported average amounts of N_2 fixed in herbaceous shrubs and tree legumes to be 8 to 142 kg ha^{-1} 9 months after planting.

The annual N_2 fixed values from sea buckthorn and buffaloberry (actinorhizal species) are more than those reported by (Busse, 2000) in *Ceanothus velutinus* with annual N_2 fixation

ranging from 4 to 15 kg ha^{-1} yr^{-1}. Busse et al. (2007) also reported annual N_2 fixation in Bitterbrush (*Purshia tridentata*) and mahala mat (*Ceanothus prostratus*) of 0.2 kg $ha^{-1}yr^{-1}$ and 0.3 kg ha^{-1} yr^{-1}, respectively, lower than what was recorded in this study. A study by Hendrickson and Burgess (1989) in southern British Columbia found that buffaloberry (*Shepherdia canadensis* L.) can fix an annual average N of 0.78 kg N ha^{-1} yr^{-1} in a 4-year regenerating lodge pine stand, a value lower than what buffaloberry fixed in this study.

Caragana had the highest whole plant N_2-fixed in both experiments. This shows that caragana can adapt and thrive well on the low N soil used in this experiment and contribute higher N compared to sea buckthorn and buffaloberry. The higher values in caragana shows the efficiency of the legume-*Rhizobium* association, which can yield between 110-227 kg N ha^{-1} yr^{-1} in pasture and forage legumes (Herridge et al., 2008). This observed trend can be explained by the significant positive correlation between whole plant N_2-fixed and shoot dry matter in both the natural abundance ($r = 0.84$, $P < .0001$) and ^{15}N dilution experiment ($r = 0.84$, $P = 0.0002$). There was a significant positive correlation between shoot dry matter and total N_2-fixed (Maskey et al., 2001; Abbasi et al., 2009; Unkovich et al., 2010). The observed higher N_2-fixed in caragana in the ^{15}N isotope dilution experiment could be due to higher nodule numbers. Because there was a significant correlation between nodule number and whole plant N_2-fixed as results of Sanginga et al. (1991) also showed a significant correlation ($r = 0.92$) between nodule number and N_2-fixed in *G. sepium*.

Generally, the amount of N_2 fixed in the test species are within the range of BNF potential of selected tree species for temperate agroforestry systems, i.e., 20-500 kg N ha^{-1} yr^{-1} for leguminous trees or shrubs and 24-236 kg N ha^{-1} yr^{-1} for non-leguminous trees or shrubs (Jose et al. 2004). The values of N_2-fixed in this study were also within the range of the amounts of N_2-

fixed from atmospheric N_2 in legume/grass pastures throughout the world (13 to 682 kg N ha^{-1} yr^{-1}) (Ledgard and Steele, 1992). The results from the study give an indication of the potential of the test species to meet and/or complement the N requirement of forage species.

In summary, despite the inclusion of buffaloberry, caragana and sea buckthorn in some land-use systems within Saskatchewan and their contribution to N management through BNF, little has been done to quantify their BNF capabilities within the prairies. This was partly because they were used to provide other ecological functions than N supply. This study employed the ^{15}N dilution techniques to quantify BNF capabilities in the test species. The techniques were employed because of their suitability for use in trees or shrubs either under greenhouse conditions or in farmer's field. Choke cherry of the same age as the test species was used as the reference to minimize the confounding problems related to reference species selection. Fixation in the test species ranged from 3 to >70 % in all the plant components using both ^{15}N dilution techniques. The test species have the potential to contribute between 11 to 73 kg N ha^{-1} yr^{-1}. Caragana contributed significantly higher amount of N in both experiments which supports the study hypothesis that BNF in caragana may be higher than in sea buckthorn and buffaloberry. This potential makes the test species suitable candidates for inclusion into existing agricultural and agroforestry systems within Saskatchewan to supply N to associated crops. Further studies are, however, needed to ascertain the fixation capabilities of the test species on the field as well as the effects of phosphorus, plant ontogeny, variety of species and moisture on the fixation capabilities. Inoculation of the planting materials prior to planting should be considered in future research as means of improving on nodulation in the test species.

4.0 Evaluating Nitrogen Transfer From a Caragana Shelterbelt by ^{15}N Natural Abundance Method and Its Effects on Yield and Nutrition of Triticale and Oats

4.1 Preface

Premising from the previous chapter (Chapter 3) on the fixation capability of the test tree/shrub species considered, the test species seem to fix N_2 with the potential to meeting the N requirements of forage and cool-season crops. This chapter looks into how much of the fixed N_2 is actually transferred to associated triticale and oats and how it affects yield and nutrition of these crops. Results obtained demonstrate the actual contribution of the N_2-fixing species studied in improving the yield and nutrition of the associated non-N_2-fixing species.

4.2 Abstract

Agricultural systems using mixtures of legumes and grasses can address some of the problems posed by excessive inorganic fertilizer use as the legumes fix atmospheric N_2 and subsequently transfer it to associated crops, with low probability of environmental problems. Understanding the belowground N transfer mechanisms under field conditions will enable the accurate estimation of the total N balance of these agroecosystems. Belowground N transfers in various systems have been widely researched, however, within the Prairies, there is dearth of information regarding N transfer in shelterbelt-based systems, the dominant agroforestry practice in the province. With the expectation that; N content of forage crops would decline with increasing distance from the shelterbelt, and access to N from the shelterbelt would increase the nutritional quality of triticale and oats, a field experiment established in the 2011 and 2012 growing seasons at the experimental site of the Agroforestry Development Centre, Indian Head,

SK, Canada. The objectives were to determine the amount of N transferred from a caragana shelterbelt to cereal species (triticale and oats) using the ^{15}N natural abundance technique and to assess the effects of this transfer on yield and forage quality. There were significant differences in the % N from fixation and N transferred based on proximity of the forage crops to the shelterbelt. Plants close (2 m) to the shelterbelt row had more % N and actual N transferred than those further away. The range of % N transferred was 8 to 64 % and 16 to 70 % to triticale and oats, respectively. This contributed to amounts of N transferred of between 0.73 to 3.20 g N m^{-2} and 1.12 to 2.28 g N m^{-2} per growing season for triticale and oats, respectively. The N contribution of the shelterbelt to the crops was close to the optimum N requirement of the species. This result implies that the N requirements of the forage species may be met by the N from the caragana shelterbelt. However, apart from total N (TN) and crude protein (CP), there were generally no significant differences ($P > 0.05$) in the measured forage quality parameters in triticale and oats based on distance from the shelterbelt row. The experiment demonstrated that the amount of N transferred belowground from the caragana shelterbelt was sufficient to meet the N requirement of triticale and oats even at 15 m away from the caragana shelterbelt.

4.3 Introduction

Despite the fact that nitrogen (N) is among the most abundant elements on earth, it is the major element limiting the growth of plants in many ecosystems due to its unavailability (Vance, 2001). To remedy this problem, N fertilizers have been used extensively as alternative sources of N to reduce yield loss, but this has contributed substantially to environmental pollution (Vance, 2001; Deustch et al., 2006). Excessive use of N fertilizers affects the balance of the N cycle in soils, contributes to eutrophication due to NO_3^- leaching and contributes to global warming due

to N_2O (Fustec et al., 2010). The energy requirements for producing N fertilizer through the Haber-Bosch process further contribute to environmental pollution and global warming.

While not a new idea, focus is returning to using N_2-fixing species especially in low-input land-use systems. This is because the BNF process can act as a sustainable source of N which can replace and/or complement N fertilizer inputs (Fustec et al., 2010). Nitrogen-fixing species in low-input land-use systems can contribute to the sustainability of the system by restoring and maintaining the soil fertility through adequate supply of N (Dulormne et al., 2003; Oenema et al., 2009).

The fixed N_2 can become available to associated crops through the process of N rhizodeposition and subsequent uptake by associated plants (Jensen, 1996). The N transfer process can either be by direct or indirect pathways, belowground and aboveground (Fig. 4.1). The main belowground pathways include: release of N by living, as well as dead and decomposed roots and nodules, and through the exudation of low molecular weight N-rich compounds from living roots which are subsequently taken up by the associated non-fixing plant (Paynel et al., 2001). Nitrogen can also be transferred belowground through mycorrhizal fungi linking the roots of associated plants via a common mycorrhizal network (CMN) (Fig. 4.1) (Johansen and Jensen, 1996; He et al., 2003). Transfers by leaf litter, root and nodule decomposition accounts for only one-third of the observed belowground transfers (Dulormne et al., 2003). Therefore understanding of other belowground N transfer pathways should lead to more efficient management of agroforestry systems (Jalonen et al., 2009). Aboveground transfer also occurs via the atmosphere through NH_3 exchange or through the urine and feces of grazing herbivores (Janzen and Gilberston, 1994; Høgh-Jensen and Schjoerring, 2000).

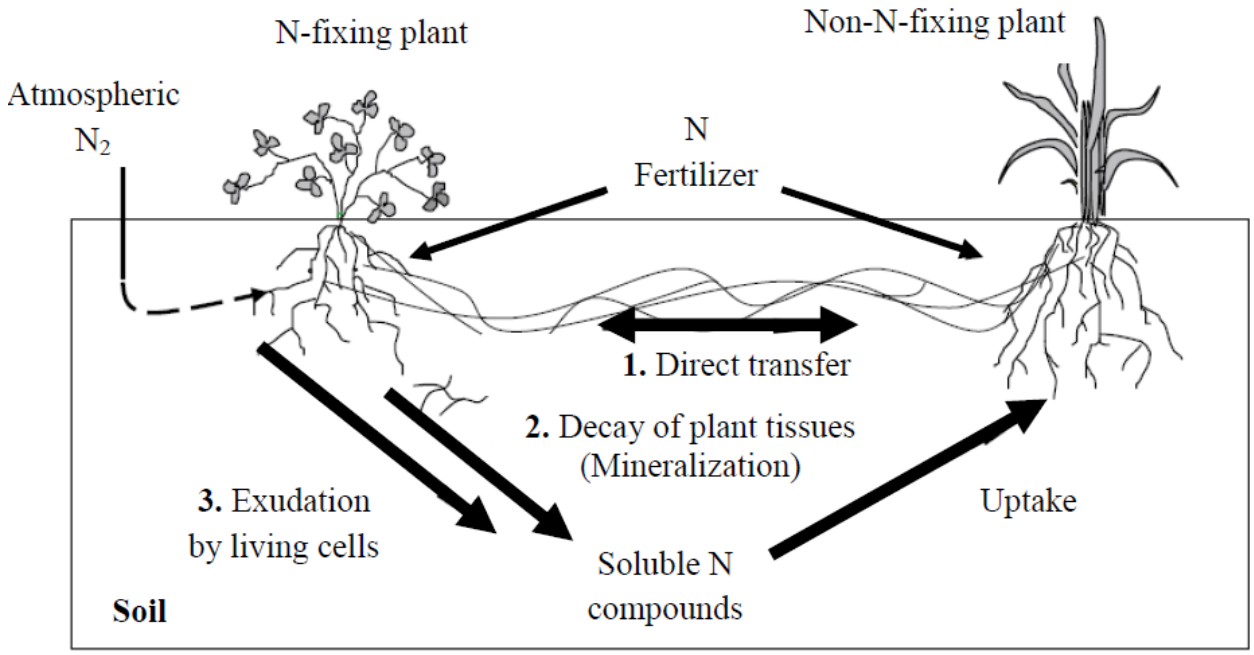

Fig. 4.1. Possible pathways of nitrogen transfer between N_2-fixing plants and non-N_2-fixing plants. 1. Direct transfer through mycorrhizal hyphae 2. Degradation and decay of N_2-fixing plants' dead tissues 3. Nitrogen exudation by living N_2-fixing plant cells (*Modified from Paynel et al., 2008*).

Nitrogen transfer from an N_2-fixing species to a companion non-N_2-fixing species can contribute substantially to meeting the N requirements of the non-N_2-fixing plants with reported estimates ranging from 0 to 68 % of the N content in the companion crop (Høgh-Jensen and Schjoerring, 1994; Rasmussen et al., 2007). It is estimated that up to 40 kg N ha^{-1} can be transferred from a legume to a grass, increasing the N content in the grass component in a mixture (Martins and Cruz, 1998). This suggests that the N requirement of grasses grown in legume-grass mixtures was met, in part, by transfer of symbiotically fixed N_2 from legumes to non-legumes (Walley et al., 1996).

The practice of adding ^{15}N fertilizer to the soil or any growth medium– ^{15}N enrichment technique is widely used to estimate BNF (Chalk and Ladha, 1999) and also has been widely employed in estimating N transfer in field-based studies. However, the method has several

limitations including: 1) plants preferring either NO_3^- or NH_4^+ as the inorganic N source (Schimann et al., 2008) 2) leaching of NO_3^- from applied ^{15}N fertilizer into deeper soils and groundwater, hence the best solution is to label with NH_4^+ which normally sorbs on soil particles and releases NO_3^- slowly through the nitrification process (Paul and Clark, 1996). Another method which relies on the inherent $\delta^{15}N$ enrichment of the soil– ^{15}N natural abundance is employed extensively in BNF estimation (He et al., 2003) but has seen limited application in N transfer studies. For example studies using the ^{15}N natural abundance method in the glacial firelands of the Athabasca Glacier, Canada showed that non-N_2-fixing plants and nearby non-nodulated actinorhizal *Dryas* plants had similar foliar $\delta^{15}N$ (Kohls et al., 1994). However, nodulated *Dryas* and nearby non-N_2-fixing plants had similar $\delta^{15}N$ values to atmospheric N_2. This suggests the transfer of symbiotically fixed N_2 from the N_2-fixing *Dryas* to the adjacent non-N_2-fixing plants (Kohls et al., 1994). Also in agroecosystems, $\delta^{15}N$ values in non-N_2-fixing understory species were similar to those of older N_2-fixing *Leucaena leucocephala,* indicating that some symbiotically fixed N_2 was transferred from the fallen biomass of *Leucaena leucocephala* to the understory species following decomposition and incorporation (van Kessel et al., 1994).

Studies conducted in agroforestry systems using the ^{15}N natural abundance technique showed that associated fodder grass *D. aristatum* crops gained substantial amounts of N from the leguminous tree *G. sepium* accounting for 4–14% of grass total N at the end of the 10 week experiment (Jalonen et al., 2009). In several coffee- legume (N_2-fixing) tree associations, 13 to 42 % of N in the coffee was from the legume trees (Snoeck et al., 2000) and 31 to 35 % of N in tropical fodder grass *Dichantium aristatum* (Poir) in association with *Gliricidia sepium* (Jacq.) was of atmospheric origin (Sierra and Nygren, 2006; Daudin and Sierra, 2008).

There are several factors that can have influence on the amount of N transferred from N_2-fixing plants to companion non-N_2-fixing plants. These factors include: biomass ratio between the donor and companion species, root turnover rate and C allocation within the species (Rasmussen et al., 2007). Other factors include the extent of nodule turnover, the size of the root system through their influence on the amounts of rhizodeposited N, soil microorganisms (i.e. the incidence of mycorrhizal fungi) and the proximity of plant root systems (Moyer-Henry et al., 2006; Phillips et al., 2006; De Graaff et al., 2007).

Understanding the mechanisms of interspecific N transfer as well as the factors that affect the transfer processes can form a basis for proper management decisions to increase productivity of legume-forage mixtures. This can lead to increased productivity of mixed forage and cereal-pulse mixtures without increasing the use of synthetic N fertilizers (Tomm et al., 1994). Nitrogen transfer in herbaceous species and the least distance between the N_2-fixing plants and non-N_2-fixing plants within which N transfer occurs have been studied extensively (Gebhart et al., 1993; Paynel and Cliquet, 2003). However, within the Prairies, there is a dearth of information on studies considering the transfer of N between N_2-fixing trees and non-N_2-fixing companion crops and the effects of distance between the associated species on N transfer. With evidence showing interplant N transfer decreasing rapidly with increasing distance between plant root systems (Hamel, 1990), this study hypothesized that: 1) N content of forage crops would decline with increasing distance from the shelterbelt, 2) access to N from the shelterbelt would increase the nutritional quality of triticale and oats. To test the hypotheses, an experiment was carried out to determine the percentage of N transferred and the amounts of N transferred to triticale (*Triticale hexaploide* Lart.) and oats (*Avena sativa* L.) from caragana (*Caragana arborescens* Lam.) and its effects on their yield and nutrition.

4.4 Materials and Methods

4.4.1 Experimental design and set-up

The experiment was set up close to a caragana shelterbelt at the experimental site of the Agroforestry Development Centre, Indian Head, SK (50^{o} 33' N, 103^{o} 39' W) as in Fig. 4.2 with distance as a continuous variable with four replications. Individual plots were 15 m × 20 m with individual sampling points 0.60 m × 0.60 m at distances 2 m, 4 m, 6 m, 15 m and 20 m from the caragana shelterbelt (Fig. 4.2). The caragana shelterbelt (ca. 5 m high) on the nursery site (50.502867°, -103.697232°) was planted in 1933 (ca. 80 years) in the North-South orientation.

The site being a nursery site was intensively managed with major cultural practices including; irrigation, fertilization, and herbicide and pesticide application. The site has been under nursery crops rotated with cereals and legumes as green manures. The soil on the site is Orthic Oxbow with a landform described as loamy morainal and hummocky, with a slope of 2-5% (Saskatchewan Soil Survey, 1986). In the 2011 growing season –when triticale was seeded, the site received a total precipitation of 102.20 mm. During the 2012 growing season, oats was seeded and the site received a total precipitation of 285.40 mm (Government of Canada, 2013).

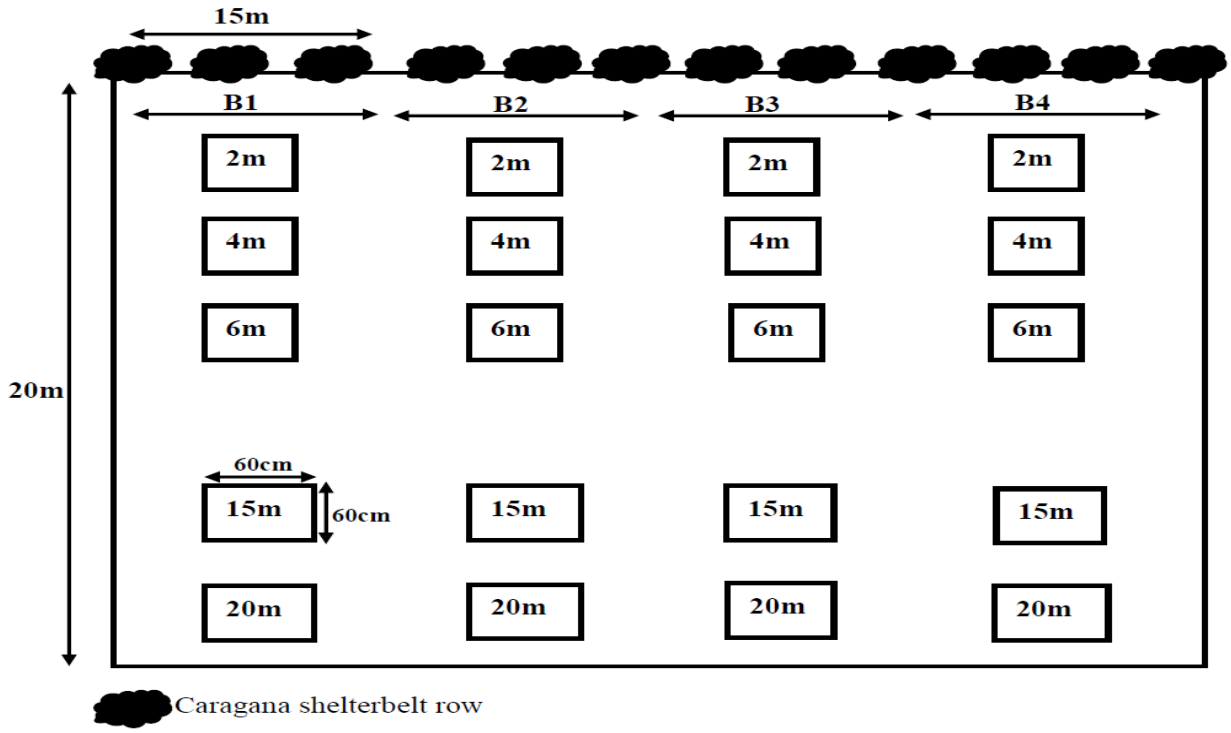

Fig. 4.2. Diagram (not drawn to scale) of experimental plot layout showing replicates and sampling subplots at 2 m, 4 m, 6 m, 15 m and 20 m from the caragana shelterbelt (60 cm × 60 cm) within each 15 m swath.

4.4.2 Initial soil sampling and analysis

Prior to setting up the experiment, the soil was sampled in three depth increments (0-15cm, 15-30 cm and 30-45 cm) at random locations within the entire experimental plot for chemical analysis to characterize the site (Table 4.1). Soil samples were also taken in three depth increments (0-15cm, 15-30 cm and 30-45 cm) at five distances (2 m, 4 m, 6 m, 15 m and 20 m) away from the caragana shelterbelt to check trend of soil nutrient with distance from the tree line (Table 4.2). All the soil samples were analyzed for total inorganic N (NO_3^- and NH_4^+) after extraction with 2*M* KCl solution (Maynard et al., 2008) and available phosphorus (P) by using the modified Kelowna method (Qian et al., 1994). Extracts were analyzed colorimetrically using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA). Exchangeable Ca^{2+}, K^+ and Mg^{2+} were analyzed following extraction with 1*M* ammonium

acetate solution using an atomic absorption spectrometer (AAS) (Varian SpectrAA 220, Burladingen, Germany) (Hendershot et al., 2008). Available sulphur (S) was determined on an autoanalyzer following 0.01*M* $CaCl_2$ extraction (Hu et al., 2005). Organic carbon (OC) was determined by dry combustion using a LECO C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA). pH and electrical conductivity (EC) (1:2 soil:water, Hendershot et al., 2008) were determined using a Beckman 50 pH Meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA), respectively.

Table 4.1. Physicochemical properties of Orthic Oxbow soil at the experimental site of the Agroforestry Development, Indian Head, SK.

Parameter	Sampling Depth		
	0-15 cm	15-30 cm	30-45 cm
pH (1:2 soil:water)	8.03†	8.32	8.73
Electrical conductivity (1:2 soil:water) (mS cm^{-1})	0.36	0.33	0.41
Available phosphorus (mg kg^{-1})	34.20	11.30	10.70
Available inorganic N (NO_3^-) (mg kg^{-1})	7.20	1.80	1.50
Available inorganic N (NH_4^+) (mg kg^{-1})	2.30	3.00	4.90
Available sulphur (S) (mg kg^{-1})	11.37	9.23	11.44
Organic carbon (OC) (g kg^{-1})	18.00	11.20	6.85
Exchangeable K^+ (cmol (+) kg^{-1})	0.91	0.43	0.33
Exchangeable Mg^{2+} (cmol (+) kg^{-1})	2.41	2.98	4.66
Exchangeable Ca^{2+} (cmol (+) kg^{-1})	9.06	9.16	7.48

†Mean values (n = 4)

Table 4.2. Physicochemical properties of Orthic Oxbow soil at the experimental site of the Agroforestry Development Centre, Indian Head, Saskatchewan based on sampling depth and distance from the caragana shelterbelt.

Distance (m)	pH	EC (mS cm^{-1})	P	NO_3^-	NH_4^+	S	OC	K^+	Mg^{2+}	Ca^{2+}
			.....mg kg^{-1}.....				g kg^{-1}	.....cmol (+) kg^{-1}.....		
					0-15 cm					
2	8.04†	0.26	22.04	8.27	3.72	5.66	7.55	0.70	1.86	9.83
4	8.05	0.17	26.21	2.40	3.09	5.15	7.05	0.62	1.66	9.29
6	8.06	0.19	21.34	1.52	2.85	4.47	7.00	0.52	1.98	9.50
15	8.01	0.22	27.23	2.23	3.36	7.65	7.20	0.63	2.21	7.75
20	7.93	0.26	31.94	2.99	3.77	9.00	7.45	0.65	2.44	7.27
					15-30 cm					
2	8.21	0.20	6.40	1.50	4.27	4.18	4.50	0.36	2.28	9.07
4	8.31	0.16	6.31	1.02	3.74	4.25	5.10	034	2.06	9.32
6	8.37	0.15	11.48	1.25	3.85	4.29	4.95	0.33	2.11	8.86
15	8.17	0.20	9.50	1.17	3.32	6.55	5.90	0.34	2.35	7.79
20	8.26	0.23	8.51	0.87	3.07	11.16	5.15	0.29	2.91	8.21
					30-45 cm					
2	6.40	0.13	5.36	0.22	3.33	3.37	2.45	0.23	2.58	6.13
4	8.49	0.18	9.77	0.29	3.94	4.18	3.00	0.31	3.10	8.34
6	8.45	0.20	6.09	0.67	3.66	4.11	3.55	0.24	2.70	8.27
15	8.44	0.21	5.64	0.49	3.30	7.80	3.45	0.23	2.75	7.90
20	8.56	0.29	5.16	0.46	4.25	nd‡	3.80	0.25	3.34	7.79

†Mean values (n = 4), ‡ nd = no data

4.4.3 Plant establishment and sampling

On July 7, 2011, AC Ultima, a spring triticale cultivar (*X Triticosecale* Wittmack) was seeded at a rate of 90 kg ha^{-1} adjacent to an existing 5 m high caragana shelterbelt. The triticale was planted 2 m away from the middle of the caragana row. On September 15, 2011, about nine weeks from the date of planting, the triticale in the 60 cm × 60 cm sampling plot was harvested 5 cm above the soil surface with a pair of scissors. In mid-May 2012, Pinnacle oats (*Avena sativa* L.) was seeded at a rate of 90 kg ha^{-1}. In the first week in August, about ten weeks from the date of planting, oats samples in the 60 cm × 60 cm sampling plot was harvested 5 cm above the soil surface with a pair of scissors. Oats samples in a Manitoba maple-oats alley cropping plots were sampled to serve as control samples to aid in determining the % $N_{transfer}$.

4.4.4 Plant analysis

Plant tissue samples harvested were oven-dried at 60 °C to stable weight to determine dry biomass and subsequently ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ), and re-ground to fine powder using a ball grinder (8000D Mixer/Mill, SPEX Sample Prep® LLC., Metuchen, NJ, USA). The ground plant samples were weighed using a micro-balance (Sartorius Microbalance, CPA2P, Bradford, MA, USA) and encapsulated in 8 mm × 5 mm tin capsules for analysis of N concentration and $\delta^{15}N$ on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany) at the University of Saskatchewan (Saskatoon, SK, Canada). The $\delta^{15}N$ of the samples was used to calculate the percentage of N derived from the atmosphere (% Ndfa) that was transferred from the caragana to the triticale and oats (% $N_{transfer}$) (Sierra et al., 2007):

$$\%N_{transfer} = \left(\frac{\delta^{15}N_{crop} - \delta^{15}N_{tree/crop}}{\delta^{15}N_{crop} - \delta^{15}N_{tree}} \right) \times 100\% \quad [4.1]$$

where, $\delta^{15}N_{crop}$ is the reference value corresponding to the triticale and oats far away from the caragana shelterbelt and represents N from soil mineral; $\delta^{15}N_{tree/crop}$ corresponds to the triticale and oats influenced by the shelterbelt and assumed to access N from two N sources (i.e., soil mineral N and fixed N_2 from caragana). $\delta^{15}N_{tree}$ is the reference value for the tree N source, and represents N coming from the caragana tree. The $\delta^{15}N_{tree}$ value was determined by taking plant tissue samples from four canopy locations on random caragana trees within the shelterbelt row. The samples were composited and analyzed for $\delta^{15}N_{tree}$ (n = 4) to represent $\delta^{15}N$ from tree exudates. To calculate the actual N transferred from the caragana to the triticale and oats, the $\%N_{transfer}$ was multiplied by the grass N content.

Forage quality indices including dry matter content (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed at the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada. Dry matter content was determined according to methods of the Association of Official Analytical Chemists (ID 930.5) (AOAC, 2005); CP was determined using AOAC standard method (ID 984.13) (AOAC, 2005) where the N content was multiplied by a factor of 6.25. Neutral detergent fibre and ADF were determined using AOAC standard method (ID 973.18) (AOAC, 2005).

4.4.5 Statistical analysis

Statistical analyses were performed on % $N_{transfer}$, amount of N transferred, forage quality parameters (DM, CP, ADF and NDF), % N and N content using SAS version 9.2 (SAS, 2008). Simple linear regression was performed on % $N_{transfer}$, amount of N transferred to determine its

relationship with distance from the tree row using PROC REG procedure in SAS. Correlation analysis was performed using PROC CORR procedure in SAS (SAS, 2008) with % $N_{transfer}$, amount of N transferred and forage quality as parameters of interest against distance. Polynomial regression analysis was also performed to identify the kind of relationship existing between forage quality indices (DM, CP, ADF and NDF), % $N_{transfer}$ and amount of N transferred.

4.5 Results

4.5.1 Contribution of caragana to the nitrogen economy of triticale and oats

Except % $N_{transfer}$ and amount of N transferred (g m^{-2}) in oats at 2 m from the caragana row in the 2012 growing season (Figs. 4.4A and 4.4B)., both % $N_{transfer}$ and the amount of N transferred (g m^{-2}) generally decreased with increasing distance from the caragana shelterbelt (Figs. 4.3 and 4.4). Percentage of $N_{transfer}$ and amount of N transferred in triticale in 2011 showed that there was a general decline with increasing distance from the caragana shelterbelt (Fig. 4.3). In 2012, oats 2 m from the caragana had low % $N_{transfer}$ and N transferred but from 4 m to 20 m there was a general decline with increasing distance (Fig. 4.4A and 4.4B). The general trend in % $N_{transfer}$ and amount of N transferred (g m^{-2}) within 4 m from the shelterbelt row were higher than corresponding values at 20 m away. Simple linear regression showed that there was a negative linear relationship between both % $N_{transfer}$ and actual amount of N transferred, and distance from the caragana row in triticale (Fig. 4.3) and oats (Fig. 4.4) in the 2011 and 2012 growing seasons, respectively.

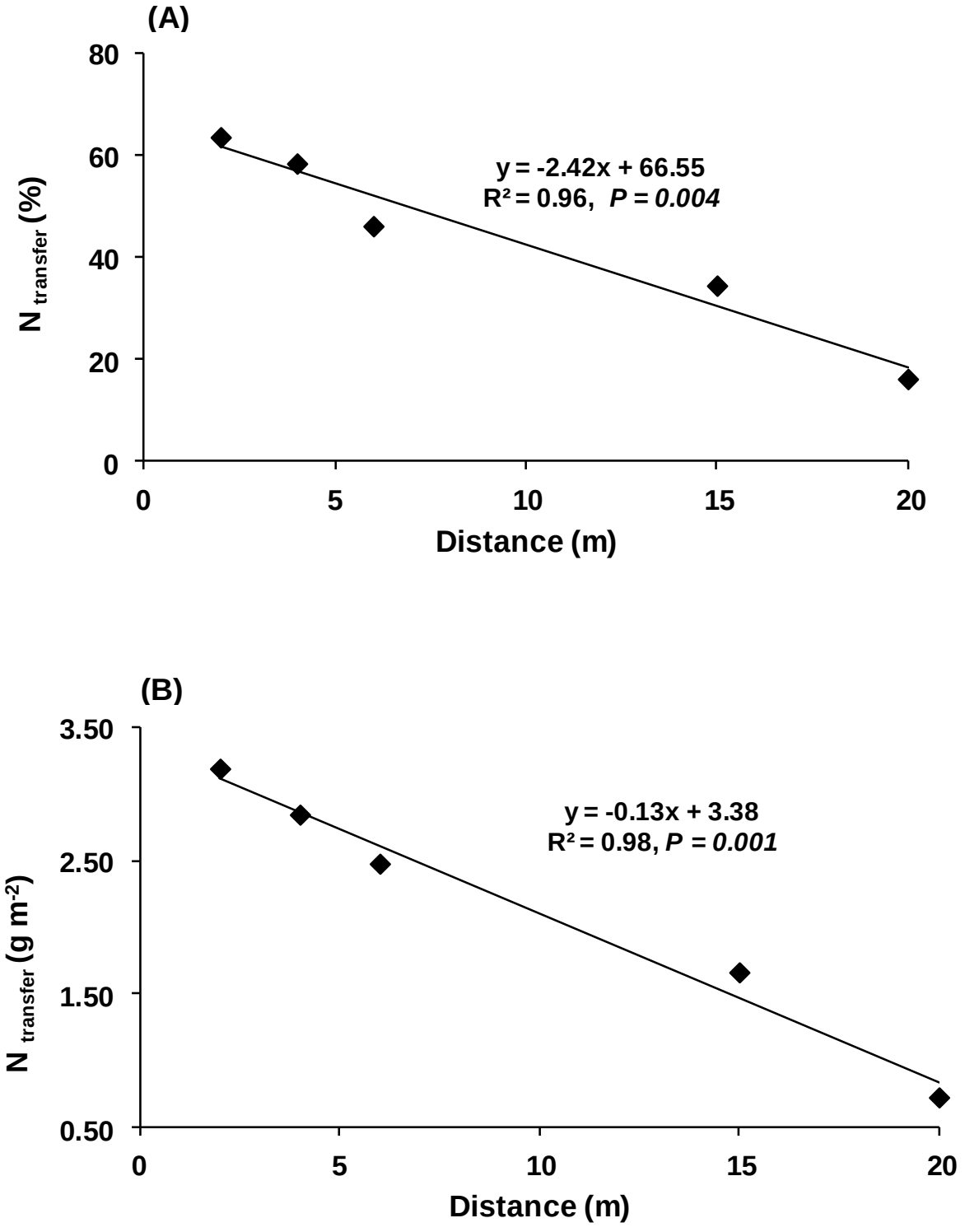

Fig. 4.3. Relationship between $N_{transfer}$ (%) and distance (A), $N_{transfer}$ (g m^{-2}) and distance (B) in triticale at a caragana shelterbelt at Indian Head in the 2011 growing season (n = 4).

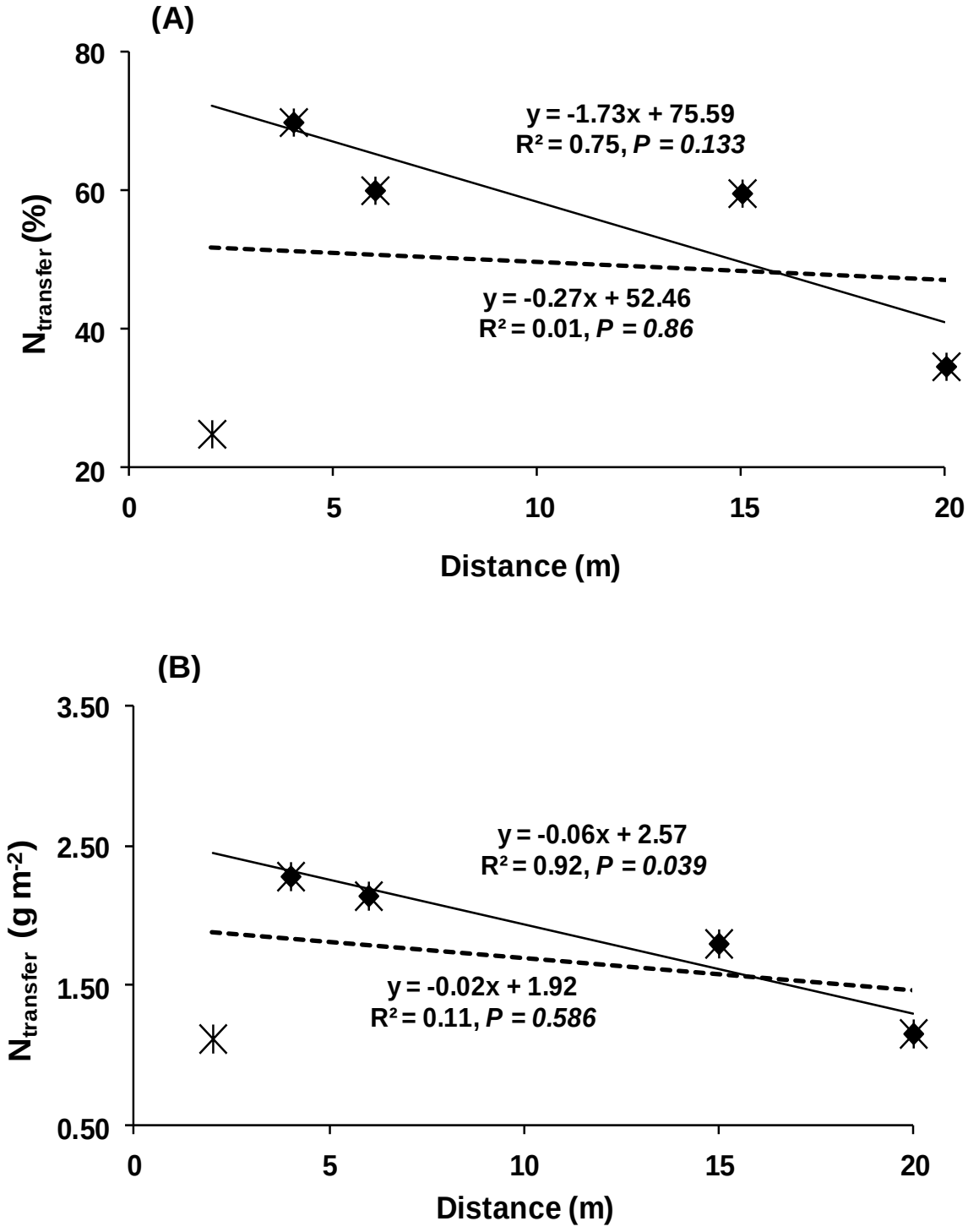

Fig. 4.4. Relationship between $N_{transfer}$ (%) and distance (A), $N_{transfer}$ (g m^{-2}) and distance (B) in oats at a caragana shelterbelt at Indian Head in the 2012 growing season (n = 4). Asterisk (*) and broken lines represent five treatments (2m, 4m, 6m, 15m and 20m), diamond (♦) and solid lines represent four treatments (4m, 6m, 15m and 20m).

4.5.2 Effects of caragana on forage quality of triticale and oats

Despite the general decline in % $N_{transfer}$ and the amounts of N transferred (g m^{-2}) with the various distances from the caragana row, there were no significant differences in CP (P = 0.2303) and ADF (P = 0.0508) based on the proximity of triticale from the caragana row (Fig. 4.5). However, NDF (P = 0.0006) generally decreased with increasing distance from the caragana row. Triticale dry matter content increased slightly from 2 m to 4 m (Fig. 4.5). Despite statistical significance (P < 0.05), the difference in DM content at the different distances was slight (93 to 95 %). In 2012, the oats samples only differed in their CP content (P <.0001) with oats 2 m from the caragana row having higher % CP than oats further away (Fig. 4.6).

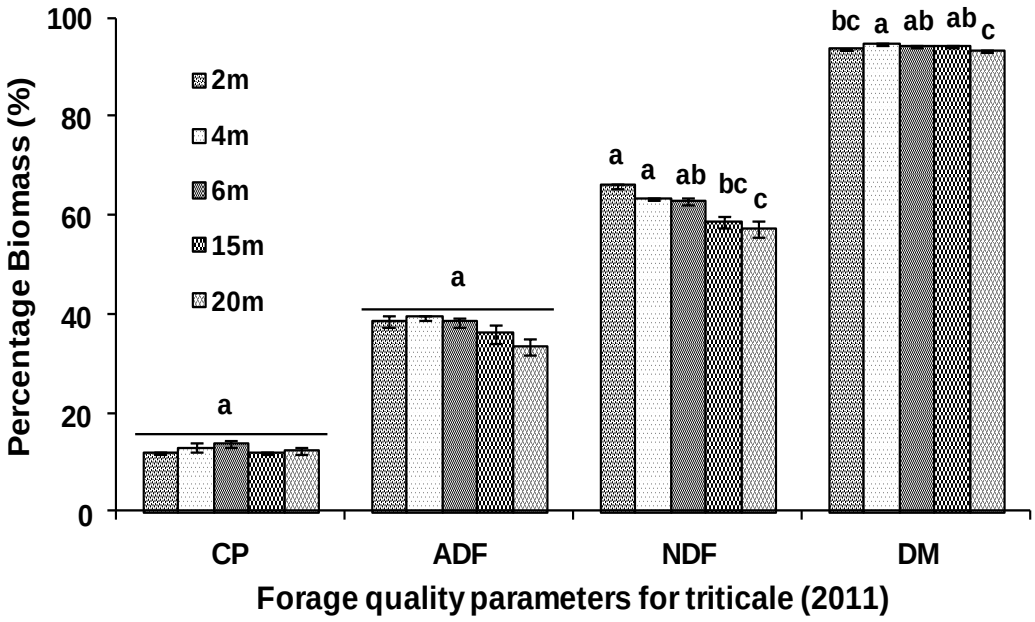

Fig. 4.5. Forage quality parameters of triticale at different distances from the caragana shelterbelt. Vertical bars show standard error of the mean (n = 4). Bars with same letters within a forage quality parameter are not significantly different at P ≤ 0.05 according to Tukey's HSD. CP = crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre and DM = dry matter content

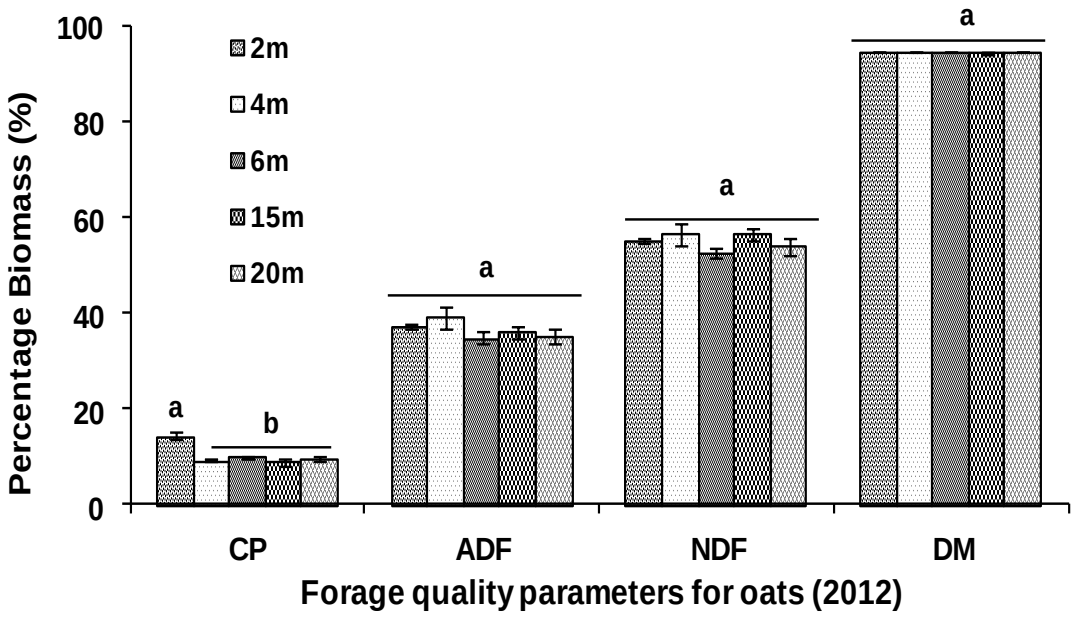

Fig. 4.6. Forage quality parameters in oats at different distances from the caragana shelterbelt. Vertical bars show standard error of the mean (n = 4). Bars with same letters within a forage quality parameter are not significantly different at $P \leq 0.05$ according to Tukey's HSD. CP = crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre and DM = dry matter content.

4.5.3 Relationship between nitrogen transfer and other parameters

Correlation analysis showed that there was a significant positive correlation between % $N_{transfer}$ and amounts of N transferred (g m^{-2}) in triticale (r = 0.98, *P* <.0001) and oats (r = 0.97, *P* <.0001) in the 2011 and 2012 growing seasons, respectively (Tables 4.3 and 4.4). In the 2011 growing season, there was a significant correlation between % $N_{transfer}$ and ADF (r = 0.56, *P* = 0.0308), and % $N_{transfer}$ and NDF (r = 0.56, *P* = 0.0303) in triticale. Amount of N transferred (g m^{-2}) in triticale was also significantly correlated with ADF (r = 0.55, *P* = 0.0324) and NDF (r = 0.57, *P* = 0.0250) (Table 4.3). In 2012, there was a significant negative correlation between % $N_{transfer}$ and CP (r = -0.64, *P* = 0.0023) and amount of N transferred (g m^{-2}) and CP (r = -0.53, *P* = 0.0166) in oats (Table 4.4). There was a significant negative correlation between N concentration and % $N_{transfer}$ (r = -0.65, *P* = 0.0019) (Table 4.4).

Results of polynomial regression (Table 4.5) showed that CP was significant in the overall regression model in both triticale (R^2 = 0.78, *P* = 0.0120) and oats (R^2 = 0.88, *P* <.0001) in the 2011 and 2012 growing seasons, respectively. However, in both growing seasons, there was neither linear nor quadratic relationship between $N_{transfer}$ (%) nor the actual N transferred (g m^{-2}) with all other forage quality parameters.

Table 4.3. Relationship among % $N_{transfer}$, amount of nitrogen transferred and forage quality parameters in triticale from caragana shelterbelt in the 2011 growing season, DM = dry matter content, CP = crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre, %$N_{transfer}$ = N transfer (%), N transferred = N transfer (gm^{-2}) and %N = N percentage.

	DM	CP	ADF	NDF	% $N_{transfer}$	N transferred	% N
CP	0.353† 0.197‡	1.000					
ADF	0.602 0.018*	0.109 0.700	1.000				
NDF	0.230 0.409	0.139 0.620	0.786 0.0005***	1.000			
% $N_{transfer}$	0.314 0.254	-0.127 0.651	0.558 0.031*	0.559 0.030*	1.000		
N transferred	0.333 0.225	0.000 1.0000	0.553 0.032*	0.575 0.025*	0.984 <.0001***	1.000	
% N	0.071 0.801	0.791 0.0004**	0.034 0.904	0.157 0.577	-0.048 0.866	0.115 0.684	1.000
N conc.	0.124 0.659	0.805 0.0003**	0.065 0.819	0.167 0.551	-0.031 0.912	0.132 0.6397	0.998 <.0001***

***** = significant at 5% level of significance, ** = significant at 1 % level of significance and *** = significant at 0.1%.
† Correlation coefficient
‡ *P* values

Table 4.4. Relationship among % $N_{transfer}$, amount of nitrogen transferred and forage quality parameters in oats from caragana shelterbelt in the 2012 growing season, DM = dry matter content, CP = crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre, %$N_{transfer}$ = N transfer (%), N transferred = N transfer (gm^{-2}) and %N = N percentage.

	DM	CP	ADF	NDF	% $N_{transfer}$	N transferred	% N
CP	-0.078†	1.000					
	0.743‡						
ADF	0.207	-0.040	1.000				
	0.382	0.866					
NDF	0.108	-0.203	0.877	1.000			
	0.650	0.390	<.0001***				
% $N_{transfer}$	-0.079	-0.643	-0.129	-0.045	1.000		
	0.739	0.002**	0.589	0.852			
N transferred	-0.123	-0.528	-0.217	-0.170	0.969	1.000	
	0.605	0.017*	0.357	0.473	<.0001***		
% N	0.013	0.983	-0.070	-0.258	-0.650	-0.548	1.000
	0.957	<.0001***	0.768	0.273	0.002**	0.012*	
N conc.	0.023	0.98187	-0.069	-0.256	-0.650	-0.548	0.999
	0.924	<.0001***	0.773	0.276	0.002**	0.012*	<.0001***

***** = significant at 5% level of significance, ** = significant at 1 % level of significance and *** = significant at 0.1%.
† Correlation coefficient
‡ *P* values

Table 4.5. Polynomial models for forage quality parameters as influenced by % $N_{transfer}$ (NT) and amount of N transferred (NTD) from caragana shelterbelt in triticale and oats for the 2011 and 2012 growing seasons.

Parameter	Polynomial model	R^2	*P*
	Triticale		
Dry Matter Content (DM)	$Y = 93.091 + 0.0350\ NT - 0.00051\ NT^2 + 0.0089\ NTD - 0.00001\ NTD^2$	0.23	0.6233
Crude Protein (CP)	$Y = 11.280 - 0.3631\ NT + 0.00181\ NT^2 + 0.2491\ NTD - 0.00089\ NTD^2$	**0.73**	**0.0120***
Acid Detergent Fibre (ADF)	$Y = 30.559 + 0.7072\ NT - 0.00707\ NT^2 - 0.2474\ NTD + 0.00151\ NTD^2$	0.47	0.1746
Neutral Detergent Fibre (NDF)	$Y = 57.753 + 1.0901\ NT - 0.01196\ NT^2 - 0.4510\ NTD + 0.00308\ NTD^2$	0.54	0.1022
	Oats		
Dry Matter Content (DM)	$Y = 94.400 + 0.0091\ NT - 0.00008\ NT^2 - 0.0006\ NTD + 0.000006\ NTD^2$	0.12	0.7809
Crude Protein (CP)	$Y = 14.438 - 0.4226\ NT + 0.00233\ NT^2 + 0.0208\ NTD - 0.00062\ NTD^2$	**0.88**	**<.0001****
Acid Detergent Fibre (ADF)	$Y = 39.845 + 0.2037\ NT - 0.00004\ NT^2 - 0.2825\ NTD - 0.00085\ NTD^2$	0.20	0.5360
Neutral Detergent Fibre (NDF)	$Y = 57.441 + 0.3284\ NT - 0.00055\ NT^2 - 0.3327\ NTD + 0.00070\ NTD^2$	0.37	0.1669

*** =** significant at 5% level of significance ** = significant at 1% level of significance *** = significant at 0.1% level of significance

4.6 Discussion

4.6.1 Contribution of caragana to the nitrogen economy of triticale and oat

The general trend of % $N_{transfer}$ from the caragana row to triticale and oats was that of decreasing with increasing distance away from the shelterbelt row. Results showed that there was an increase in N in oats and triticale samples up to 15 m away from the caragana shelterbelt; hence there was a beneficial effect of associating caragana with triticale and oats. This shows that the transfer of N from caragana to triticale and/oats may either be due to intimate contact between roots of caragana and triticale and oats i.e. via a common mycorrhizal network or the transfer could have been due to root exudates or release of N by living and dead roots and nodules of caragana (Paynel et al., 2001; He et al., 2003). The % N from caragana is up to 64 % in triticale and 70 % in oats. These values are similar to those found by Seiter et al. (1995) who reported that up to 58 % of the total N in maize (*Zea mays* L.) was derived from red alder (*Alnus rubra* Bong.) in an alley cropping system in Oregon, U.S.A. They also found that N transfer decreased with increasing distance from trees (Seiter et al., 1995). The range of N transfer values are also similar to those found by Høgh-Jensen and Schjoerring (1994) and Rasmussen et al. (2007) who reported estimates ranging from 0 to 68 %. The amount of N transferred from the caragana shelterbelt and accessed by triticale and oats were also similar. This may be because the roots of the crops have similar nutrient acquisition patterns. The amount of N transferred in this study contributed significantly to meeting the N requirements of triticale and oats. This corroborates studies by Høgh-Jensen and Schjoerring (1994) and Rasmussen et al. (2007) who reported that N transfer from an N_2-fixing species to a companion non-N_2-fixing species can contribute substantially to meeting the N requirements of non-N_2-fixing plants.

In the 2011 growing season, the % $N_{transfer}$ to triticale ranged between 8 to 64 % which translated into 0.73 to 3.20 g N m^{-2}. Also in the 2012 growing season, the % $N_{transfer}$ ranged from

16 to 70 %, contributing between 1.12 to 2.28 g N m^{-2} to the associated oats plants. In the 2012 growing season, there was a low amount of % $N_{transfer}$ and actual N transferred to oats at the 2 m distance. At 4 m, transfer was high (70 %) following a general trend of decreasing with increasing distance. Before N transfer from a legume to a non-legume grass occurs, the growth of the grass plant should be limited by N (Hamel, 1990). Plants at 2 m from the caragana row had significantly higher CP content compared to oats further away from the caragana shelterbelt, indicating that oats plants near the shelterbelt were not N limited. This was further confirmed by the significant negative correlation between both % N and N concentration with % $N_{transfer}$ and amount of N transferred. Another possible reason for low % $N_{transfer}$ and actual N transferred could be due to competition between caragana and oats for resources such as moisture, light and other nutrients apart from N, as moisture competition was reported to decrease yields of crops near black walnut trees (Jose et al., 2000a). Despite the decrease in % $N_{transfer}$ and actual N transferred to oats 2 m from the caragana row, there was generally no effect on forage quality parameters in oats.

The overall general trend of decreasing % $N_{transfer}$ and amount of N transferred with distance in both triticale and oats may be due to the decrease in root density of caragana with increasing distance from the tree row. This might have resulted in decreased supply of N belowground to the associated triticale and oats at further distance from the shelterbelt (Moyer-Henry et al., 2006; Day et al., 2010) and may have reduced the efficiency of N transfer belowground. Estimates of N transfer from this study were based on indirect dilution of soil ^{15}N by ^{14}N from caragana and may be referred to as "apparent N transfer" (Heichel and Henjum, 1991; Jørgensen et al., 1999). It is not possible to differentiate unknown N transfer from the soil to triticale and oats plants from additional soil N made available due to stimulation of N

mineralization (Vallis et al., 1977). This limitation might have led to overestimation of the N transfer from caragana to triticale and oats using this indirect dilution of soil ^{15}N.

4.6.2 Contribution of caragana to nutritive value improvement in triticale and oats

The measured forage quality indices in both triticale and oats were generally not significantly different among the various distances except DM and NDF in triticale in 2011 and CP in oats in 2012 growing seasons. The general trend of NDF and CP was a slight decrease with distance from the caragana row. However, DM had a bell-shaped trend with the 2 m and 20 m distances having lower values and the middle three distances i.e. 4 m, 6 m and 15 m having higher values. Despite the diversity of CP content among feeds, high protein content is usually associated with high feed quality. Crude protein should not be solely used as a quality criterion without evaluating the fiber content, because as forages mature, their CP is diluted by increasing fiber content (Van Saun, 2006).

Feed materials vary widely in their DM. The forage species in this study had DM content of between 93-95 %; higher than in pastures and liquid feeds (10-25 %) (Van Saun, 2006). An increase in fibre content (ADF and NDF) leads to reduced forage digestibility and intake (McGraw et al., 2008). Neutral detergent fibre is considered a good measure of feed quality and plant maturity. Grasses with NDF values less than 50 % are termed as high quality feed and those with values greater than 60 % are termed low quality feed (Van Saun, 2006). This study had NDF values ranging from 53-66 % with only few samples above 60 %, signifying the tested forage crops were of high quality. Acid detergent fibre and NDF are good measures of feed quality and are often used to predict energy content of feeds. High values of ADF and NDF within a feed suggest low-quality feed. To maintain the quality of a feed material, the aim is less

than 35 % ADF in either legume or grass forages (Van Saun, 2006). The range of values for ADF in the present study was 33-39 %, indicating that the forage was of relatively high quality.

In summary, the study confirmed belowground N transfer from the caragana shelterbelt row to the associated triticale and oats and the applicability of the ^{15}N natural abundance technique to detect N transfers. It also showed that as far as 15 m away from the caragana row, there was an appreciable amount of N transferred to the associated triticale and oats. The general trend was decreased amounts of N transferred with increasing distance from the caragana row. Despite the significant differences in % $N_{transfer}$ in triticale and oats in both growing seasons and amount of N transferred in triticale in the 2011 growing season, there were no significant differences in the forage quality parameters measured in both growing seasons, except NDF and DM content in triticale and CP in oats. However, the range of forage quality parameter values recorded showed a relatively moderate forage quality. The study illustrates that growing caragana tree in association with cereal crops, even in alleys as wide as 20 m can help meet the N requirements of the crops through N transfer. The idea can be adopted especially in low input systems as a means to reduce the overreliance on inorganic N fertilizer usage. Also, due to the wide alleyways, farm machinery operations may be permitted.

Further studies are required to: 1) explore the various mechanisms of belowground N transfer through root studies using minirhizotrons and ^{15}N isotopic tracer techniques to determine the respective contributions of the various mechanisms at each stage of the plants' growth, 2) determine the kind of N transferred, i.e., whether inorganic N, organic N or both and whether there is coincidental carbon transfer with the N, and 3) ascertain whether the transfer is bi-directional and to find out if the transfer is affected by mycorrhizal associations and/or N_2-fixation.

5.0 BIOPHYSICAL INTERACTIONS BETWEEN AGROFORESTRY TREES AND FORAGE CROPS ON THE YIELD AND NUTRITION OF THE FORAGE CROPS

5.1 Preface

The balance between facilitation and competition for nutrients, soil moisture and light at the tree-crop interface are the principal determinants of the yield and productivity of intercropped species in typical alley cropping systems. The two preceding chapters looked into the fixation of nitrogen (N_2) (Chapter 3) and its transfer and effects on yield and nutrition of forage crops (Chapter 4). This chapter focuses on the effects of soil moisture and light on the yield and nutrition of oats intercropped within Manitoba maple alleyways. Results obtained would add to the information in the preceding chapters to understand the ameliorative effects of trees on nutrients, soil moisture and light at the tree-crop interface to influence crop production in alley cropping systems.

5.2 Abstract

Competition between trees and crops in alley cropping systems for soil moisture, light and nutrients, especially N can lead to decreased productivity of the associated crop. The objective of this study was to evaluate the biophysical interactions of soil moisture and light between Manitoba maple and oats on the yield and nutrition of the oats crop. Oats was seeded within a 9-year old established Manitoba maple alley cropping site at the experimental site of the Agroforestry Development Centre, Indian Head, SK, Canada in the 2012 growing season. Tree rows were arranged in the east/west direction and the oats plots were established on the north and south sides of the Manitoba maple rows to evaluate the effects of orientation. Gravimetric soil moisture content (SMC) and photosynthetically active radiation (PAR) were collected to

evaluate the effects of trees on SMC and PAR with reference to the tree line. Apart from crude protein (CP) and total nitrogen (TN) in the oats plant, dry matter content (DM), acid detergent fibre (ADF) and neutral detergent fibre (NDF) were not affected by distance from the tree row, orientation of oats plots and their interactions. However, oats grown in the northern orientated plots had generally higher values in all the measured forage indices compared to the oats in the southern orientation, which largely corresponded to the trend in soil moisture content. It can therefore be concluded from this study that SMC played a major role in determining the DM content and nutrition in oats more than PAR.

5.3 Introduction

Alley cropping is defined as the simultaneous growing of two or more species in the same field for a significant period of their growth (Bedoussac and Justes, 2010). It is one of the agroforestry practices recently being researched within North America (Thevathasan et al., 2004; Garrett, 2009). Despite the long-term practice of alley cropping around the globe for food, wood, browse, fruit or nuts, it has not been adopted in temperate agriculture landscapes such as Canada due to the implementation of highly mechanized monocrop agriculture, and technological and demographic changes resulting in larger farm fields (Kort et al., 2009). The practice has been more widely implemented in Europe but is largely absent from the agricultural landscape of Canada (Bergeron et al, 2011), especially within the prairie eco-region. In contrast to tropical alley cropping, temperate alley cropping has evolved to mean cropping the alleyways between rows of trees of varied width, where the trees are grown to maturity without repeated coppicing for mulch or green manure supply (Gillespie et al., 2000).

Interactions in agroforestry, in general, can be defined as the influence of one component on the performance of another component (s) of the system as well as on the overall system itself (Nair, 1993). The interactions usually occur at the tree-crop and/or tree-animal interface and can be belowground or aboveground. However, there may be instances in certain environments where the effect of the tree has no impact on the availability of a resource and performance of the system, such as when a resource is not limiting (Kho, 2000). Tree-crop interactions are not constant and may be affected by total planting densities, tree-crop combinations, climatic and soil conditions and management regimes (Imo and Timmer, 2000). Exploitation of positive interactions (i.e. facilitative and complementary) between the woody and non-woody components and the minimization of negative interactions (i.e. competitive) is key to the success of agroforestry systems.

Competitive interactions occur within the competition zone and depending on the species type, age, height and soil may occur within a 2-m distance from the tree row (Fig. 5.1). This zone is characterized by direct competition for nutrients, moisture and light (Cardinael et al., 2012). Competitive interaction also occurs if the agroforestry trees limit the use of resources from the same pool as understory plants (Fernandez et al., 2008).

The complementary zone is the zone occurring at a distance greater than 2 m from the tree row (Fig. 5.1) and is characterized by favourable growing conditions with enhanced nutrient cycling, N mineralization, soil organic carbon addition and earthworm activity (Thevathasan and Gordon, 2004; Clinch et al., 2009). The complementary zone has lower soil temperature and higher moisture availability relative to the competitive zone due to less evapo-transpiration and carbon assimilation (Thevathasan and Gordon, 2004; Reynolds et al., 2007; Clinch et al., 2009).

Belowground interactions in agroforestry systems could be facilitative via carbon enrichment, capturing of leached nutrients, and improvement in soil physical properties or supply of hydraulically lifted water. Complementary interactions can occur when deep-rooted trees use resources which are otherwise not accessible to the associated crops. This results in efficient use of resources by the association as a whole.

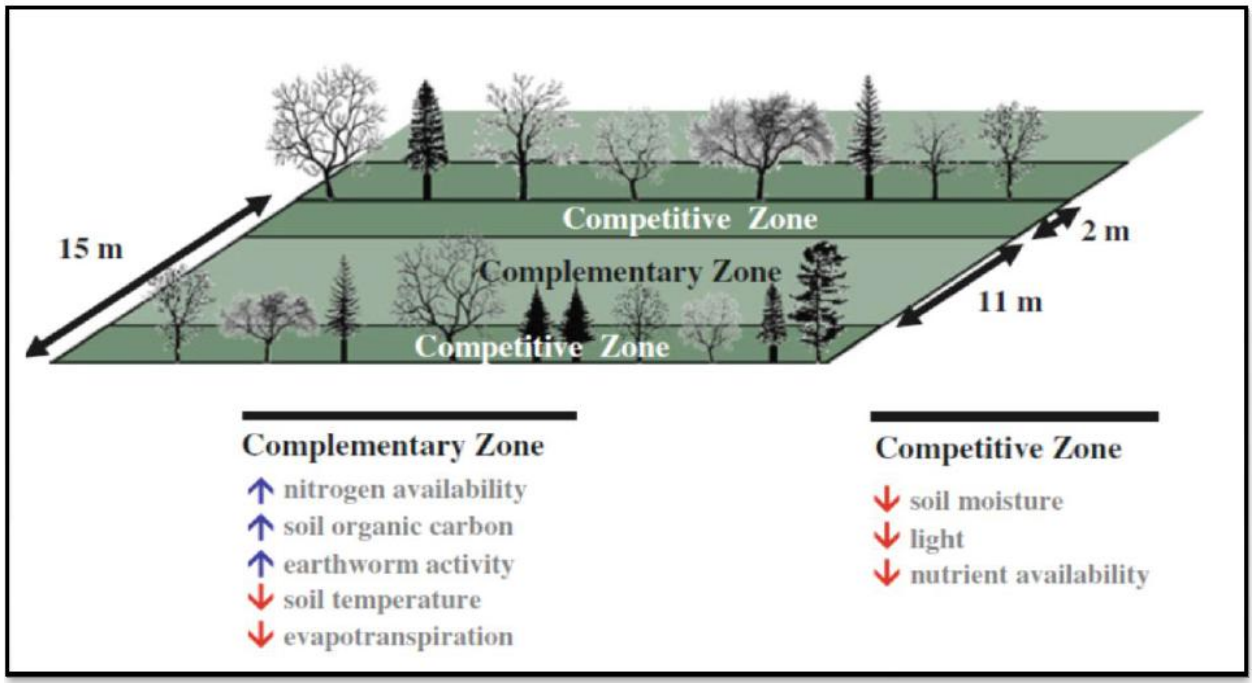

Fig. 5.1. Schematic diagram showing the 'competitive' and the 'complementary' zones in tree- based intercropping field at the Guelph Agroforestry Research Station (GARS), Guelph, ON, Canada. Blue arrows indicate increased in the parameters and red arrows indicate decreased in the parameters (*Modified from Cardinael et al., 2012*).

With the potential competition between trees and crops, it is important to minimize competitive interaction and maximize complementary interactions (Thevathasan and Gordon, 2004). This can be achieved via understanding of parameters such as the minimum distance required between intercropped tree rows and crop plants to avoid significant competition for light and nutrients (Cardinael et al., 2012).

There have been several studies on tree-crop interactions with differing results. For example an intercropping system of black pepper (*Piper nigrum* L.) and cardamom (*Elettaria cardamomum* L. Maton.) with grevillea (*Grevillea robusta* A.) and gliricidia (*Gliricidia sepium* Jacq.) showed that cardamom produced higher yield with grevillea than in natural forest. Cardamom produced 5.5 times more yield in the fourth year than the average in the area. The land equivalent ratios for black pepper and cardamom showed that pepper intercropped with grevillea produced 3.9 times yield more than in monoculture whereas cardamom intercropped with grevillea and pepper produced 2.3 times yield more than in monoculture (Reyes et al., 2009). The practice of alley cropping increased yield and grain protein concentration in spring grown crops compared to monocrops, particularly in low N input systems (Hauggaard-Nielson et al., 2003). Crop yield under trees in an intercropped system was reduced due to competition (Rao et al., 1991; Chamshama et al., 1998). Numerous studies showed that competition for water resources markedly decreased crop yields in temperate regions (Jose et al., 2000a). In semi-arid tropical alley cropping systems, belowground competition for water was the major factor causing yield reduction; however, in temperate alley cropping systems aboveground light has been suggested to be the limiting factor (Singh et al., 1989; Ong et al., 1991; Chirko et al., 1996). There is also a considerable overlap between tree and crop roots in semi-arid environments in the top 50 cm of the soil (Rao et al., 1993). This demonstrates that trees could compete with crops for both water and nutrients. However, intercropping systems have been proposed in eastern Canada as a means of improving soil quality (Bradley et al., 2008).

Despite evidence of tree root influence on associated crops in temperate regions (Miller and Pallardy, 2001), there is inadequate information on the physiology and production impacts of resource competition in alley cropping setting within the Canadian prairies. This study

hypothesized that modified light and soil moisture conditions in the alleyways would contribute to improved yield and nutrition of associated oats. The hypothesis was tested in a field experiment to determine the effects of modified light and soil moisture conditions within the alleyways on the dry matter content and forage quality indices in oats.

5.4 Materials and Methods

5.4.1 Study site descriptions

The experiment was carried out during the 2012 growing season at the experimental site of the Agroforestry Development Centre, Indian Head, SK, Canada (50^{o} 33' N, 103^{o} 39' W) at a Manitoba maple alley cropping site. The Manitoba maple trees, 3 m high were planted in the East-West orientation in 2004 (ca. 9 years) as a weed-control study and managed under alley cropping. Data from the alley cropping site have been collected since 2008.

In 2012 during the study period, the site received a total precipitation of 285.40 mm (Government of Canada, 2013). The soil type of the area is classified as Orthic Oxbow with a landform described as "loamy morainal" and hummocky with a slope of 2 to 5 % (Saskatchewan Soil Survey, 1986).

5.4.2 Experimental design and set-up

The experimental design was a factorial design with three factors laid out in randomized complete block with four replications. The factors were: 1) orientation (north and south), 2) distance from the maple tree row (i.e. 2 m, 4 m and 6 m), and 3) depth in terms of SMC (soil parameters) or time of day (light parameters). Individual blocks were 9 m × 6 m (Fig. 5.2). There were ten blocks in total (four on the north and six on the south side of the tree rows). Within each

block there were three 60 cm × 60 cm sampling points at 2 m, 4 m and 6 m from the Manitoba maple row (Fig. 5.2).

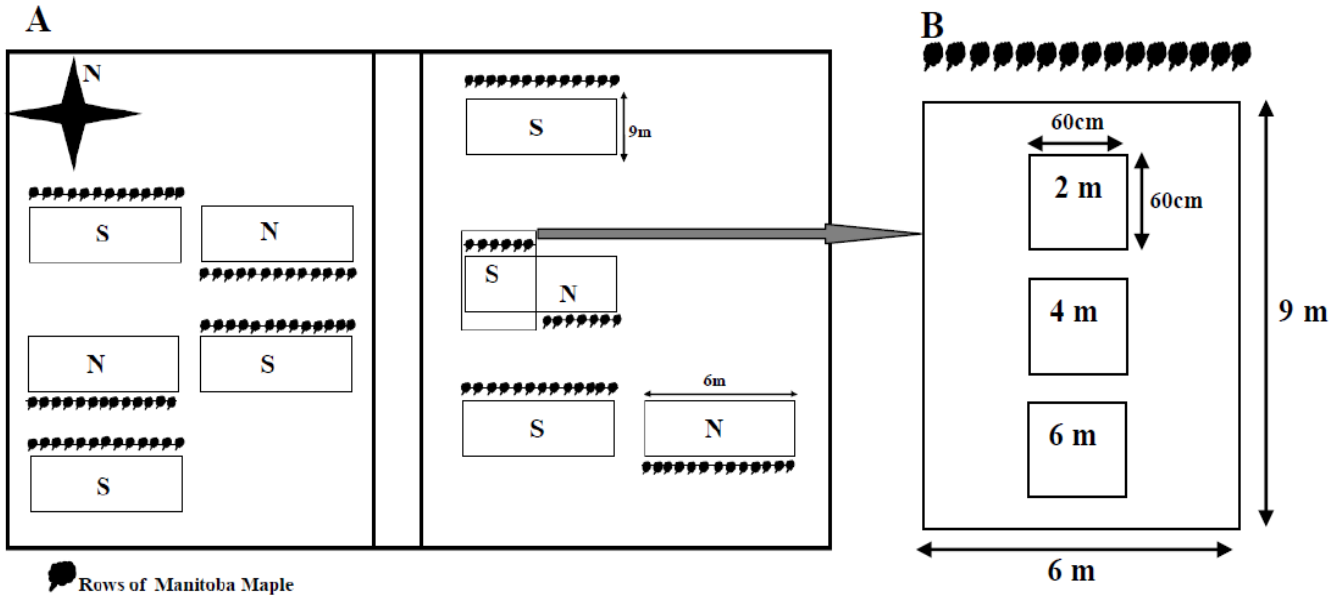

Fig. 5.2. Plot layout of field experiment, showing Manitoba maple tree rows and north and south orientation of oats plots (A). A single block with sampling plots (60 cm × 60 cm) at 2 m, 4 m and 6 m from tree line (B).

5.4.3 Soil sampling and analysis

Prior to planting the oats, the soil was sampled in three depth increments (0-15 cm, 15-30 cm and 30-45 cm) and analyzed (Table 5.1) for total inorganic N (NO_3^- and NH_4^+) after extraction with 2*M* KCl solution (Maynard et al., 2008) and available phosphorus (P) by using the modified Kelowna method (Qian et al., 1994). Extracts were analyzed colorimetrically using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA). Exchangeable Ca^{2+}, K^+ and Mg^{2+} were analyzed after extraction with 1*M* ammonium acetate solution using an atomic absorption spectrometer (AAS) (Varian SpectrAA 220, Burladingen, Germany) (Hendershot et al., 2008). Available sulphur (S) was determined by an autoanalyzer

subsequent to extraction with 0.01*M* $CaCl_2$ extraction (Hu et al., 2005). Organic carbon (OC) was determined by dry combustion using a LECO C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA). pH and electrical conductivity (EC) (1:2 soil:water, Hendershot et al., 2008) were determined using a Beckman 50 pH Meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA), respectively.

Table 5.1. The Physicochemical properties of the study site soil (Orthic Oxbow Soil) at Agroforestry Development Centre's nursery site, Indian Head, SK.

Parameter	Sampling depth		
	0-15 cm	15-30 cm	30-45 cm
pH (1:2 soil:water suspension)	8.03†	8.32	8.73
Electrical conductivity (1:2 soil:water) (mS cm^{-1})	0.36	0.33	0.41
Available phosphorus (mg kg^{-1})	34.20	11.30	10.70
Available inorganic N (NO_3^-) (mg kg^{-1})	7.20	1.80	1.50
Available inorganic N (NH_4^+) (mg kg^{-1})	2.30	3.00	4.90
Available sulphur (S) (mg kg^{-1})	11.37	9.23	11.44
Organic carbon (OC) (g kg^{-1})	18.00	11.20	6.85
Exchangeable K^+ (cmol (+) kg^{-1})	0.91	0.43	0.33
Exchangeable Mg^{2+} (cmol (+) kg^{-1})	2.41	2.98	4.66
Exchangeable Ca^{2+} (cmol (+) kg^{-1})	9.06	9.16	7.48

† Means (n = 4)

5.4.4 Oats establishment and sampling

On May 19th, 2012, Pinnacle oats (*Avena sativa* L.) was planted at a rate of 90 kg ha^{-1} within the 9-year old established alley cropping site of Manitoba maple. Blocks (9 m × 6 m)

were bound by two rows of Manitoba maple (Fig. 5.2) were established within 11-m wide rows of Manitoba maple. On August 2, 2012, at the tasseling stage, oats samples were harvested 5 cm above the soil surface with a pair of scissors in the 60 cm × 60 cm sampling plots. Thirty-four samples were collected (3 distances × 10 blocks + 4 control).

5.4.5 Plant analysis

The harvested plant tissue samples were oven-dried at 60 °C to stable weight and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Forage quality indices including dry matter content (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed at the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada. Dry matter content was determined according to methods of the Association of Official Analytical Chemists (ID 930.5) (AOAC, 2005); CP was determined using AOAC standard method (ID 984.13) (AOAC, 2005) where the N content was multiplied by a factor of 6.25. Neutral detergent fibre and ADF were determined using AOAC standard method (ID 973.18) (AOAC, 2005).

5.4.6 Microclimatic and weather parameter measurements

Gravimetric soil moisture content (SMC) in three depth increments (0-20 cm, 20-40 cm and 40-60 cm), at three distances (2 m, 4 m and 6 m) and two orientations (north and south) from the Manitoba maple rows were taken by hand using a Dutch auger bi-weekly from June to August, 2012. Sampling was normally deferred for a day or two following heavy rains.

Photosynthetically active radiation (PAR) was measured at the three designated distances from the Manitoba maple row three times per day (morning, solar noon and evening), on sunny days on a bi-weekly basis (i.e., from June-August 2012) using a Sun Scan Canopy Analysis

System equipped with a Beam Fraction Sensor (BFS) (Type SS1, Delta-T Devices Ltd., UK). This frequency of measurement was adopted because in alley cropping systems, comparative PAR measurements at mid-day provides an important yet incomplete picture of potential photosynthetic activity across the alley due to greater early- and late-day shading of border row plants by trees (Miller and Pallardy, 2001). Photosynthetically active radiation data was normally collected on sunny and clear-sky days, hence sampling was deferred almost a month after planting to coincide with clear and sunny days and continued until harvest of oats.

Relative humidity (RH) and air temperature were recorded daily from May 2012 to July 2012 using USB-500 series data loggers mounted at some locations across the experimental site (Measurement Computing Corp., Norton, MA). The recordings were done throughout the experimental period and reported on bi-weekly diurnal and nocturnal basis to represent their trend throughout the experimental period. However, due to malfunctioning of data loggers, only values up to the end of July were retrieved and reported.

5.4.7 Statistical analysis

All the parameters were normally distributed based on the Shapiro-Wilk test (SAS, 2008). Statistical analyses were performed on gravimetric soil moisture content (SMC) and photosynthetically active radiation (PAR) by REPEATED MEASURES using PROC MIXED procedure. Forage quality parameters (DM, CP, ADF and NDF) were analyzed using SAS 9.2 (SAS, 2008) using two-way analysis of variance (ANOVA) by the PROC MIXED procedure at 5 % level of significance. Blocking was a random effect factor while distance, orientation, depth and time of day were all fixed effect factors in the analysis. The SAS macro pdmix800 was used to perform pairwise comparisons at 5 % level of significance (Saxton, 1998) following significance based on Tukey's Honestly Significant Difference (HSD) in ANOVA.

5.5 Results

5.5.1 Gravimetric soil moisture content (SMC)

Analysis of variance (ANOVA) shows that both orientation and depth have effects on SMC (Appendix A Table A1). There were no differences in SMC due to the effects of distance (Fig. 5.3) (Appendix A Tables A1 & A2). Generally, there was higher SMC at all distances in the northern compared to the southern orientation. There was a tendency for lower SMC close to the tree row (2 m) compared to those within the alley (4 m and 6 m) in the southern orientation, however, the northern orientation had no marked trend (Fig. 5.3). There was a consistent decrease in SMC with soil depth (Fig. 5.4). Soil moisture content generally decreases from spring to summer (Fig. 5.7)

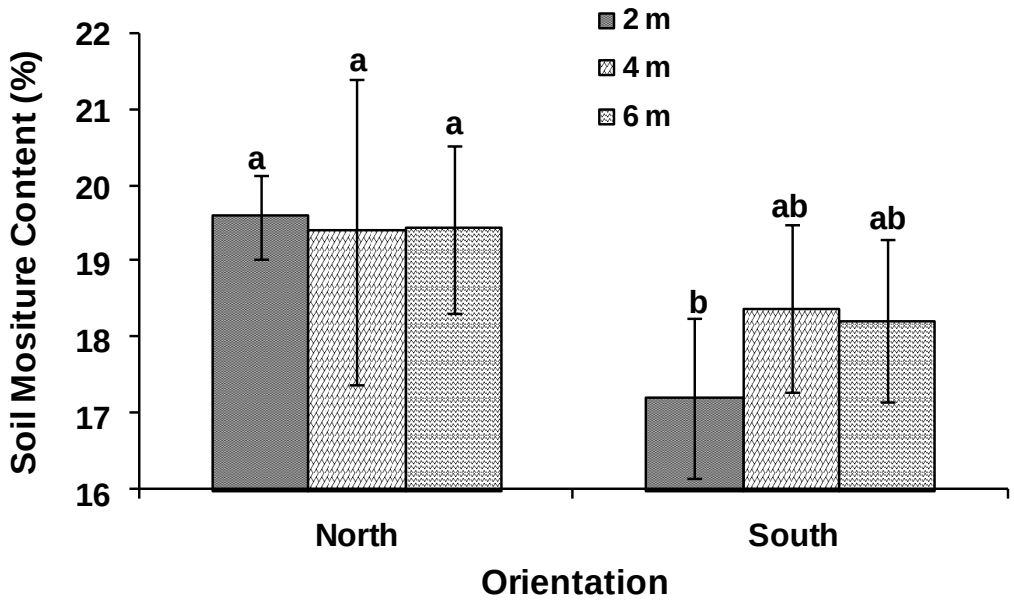

Fig. 5.3. Gravimetric soil moisture content (SMC) (%) within Manitoba maple-oats alley cropping system at Indian Head, SK. Soil samples (n = 90) were extracted from three depths (0-20 cm, 20-40 cm and 40-60 cm) bi-weekly over a three-month period within the oats plots at three distances (2 m, 4 m and 6 m) from the tree line. Plots were oriented to the north and south of the tree row. Means (all depths) followed by same letters are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

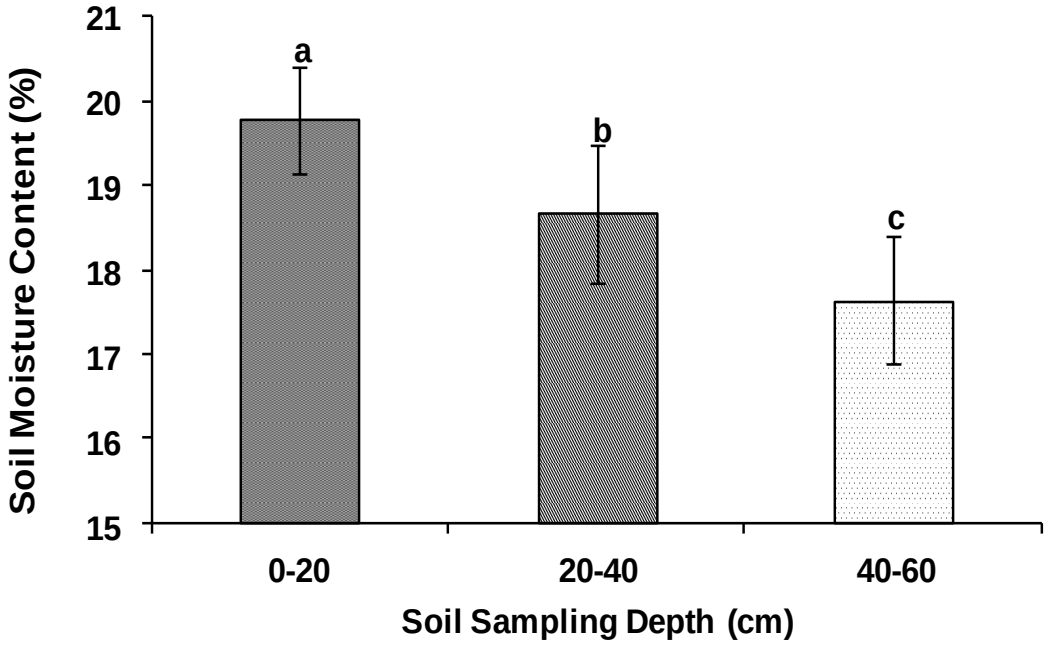

Fig. 5.4. Gravimetric soil moisture content (SMC) within Manitoba maple-oats alley cropping system at Indian Head, SK. Soil samples (n = 90) were extracted from three depths (0-20 cm, 20-40 cm and 40-60 cm) bi-weekly over a three-month period within the oats plots at three distances (2 m, 4 m and 6 m) from the tree line. Means (both orientations) followed by same letters are not significantly different at $P \leq 0.05$ according to Tukey's HSD.

5.5.2 Photosynthetically active radiation (PAR)

Orientation and distance and their interaction (P <.0001) (Fig. 5.5) affected PAR (Appendix B Tables B1 & B2). There were differences in average PAR values due to effects of time of the day (P <.0001) as well as time of day and orientation interaction (Figs. 5.6A & 5.6B). The southern orientation recorded a generally higher average combined value of PAR compared to that recorded in the northern orientation (Fig. 5.5). In the southern orientation, there were no significant differences in PAR among the distances, however, in the northern orientation PAR at 2 m was lower than PAR at 4 m and 6 m (P < .0001) (Fig. 5.5). There was also a significant difference in PAR values due to time of the day (P < .0001) with afternoon PAR values higher than the morning and evening values (Fig. 5.6A).

The range of average PAR values throughout the experiment based on distance from the Manitoba maple was ca. 400-1000 µmol m^{-2} s^{-1} in the northern orientation and ca. 900-1000 µmol m^{-2} s^{-1} in the southern orientation (Fig. 5.5) and ca. 400-1000 µmol m^{-2} s^{-1} based on time of day (Fig. 5.6B). Generally, there were higher PAR values in June/July than in August (Fig. 5.7). There was also a non-significant weak correlation between SMC and PAR (r = 0.10, *P* = 0.0570) (data not shown).

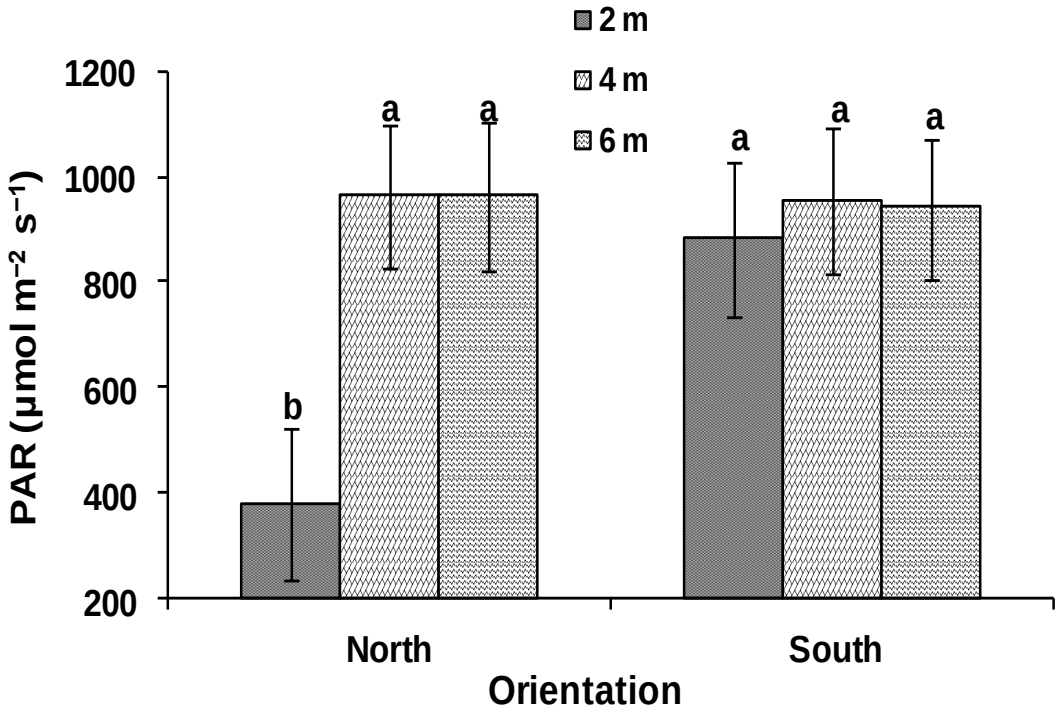

Fig. 5.5. Photosynthetically active radiation (PAR) (µmol m^{-2} s^{-1}) within Manitoba maple-oats alley cropping system at Indian Head, SK. PAR (n = 90) was done at three times of the day (morning, solar afternoon, evening) bi-weekly over a two and half-month period within the oats plots at three distances (2 m, 4 m and 6 m) from the tree line. Plots were oriented to the north and south of the tree row. Means followed by same letters are not significantly different at *P* ≤ 0.05 according to Tukey's HSD. (Morning = ca. 8:00, Solar noon = ca. 13:00 and Evening = ca. 18:00).

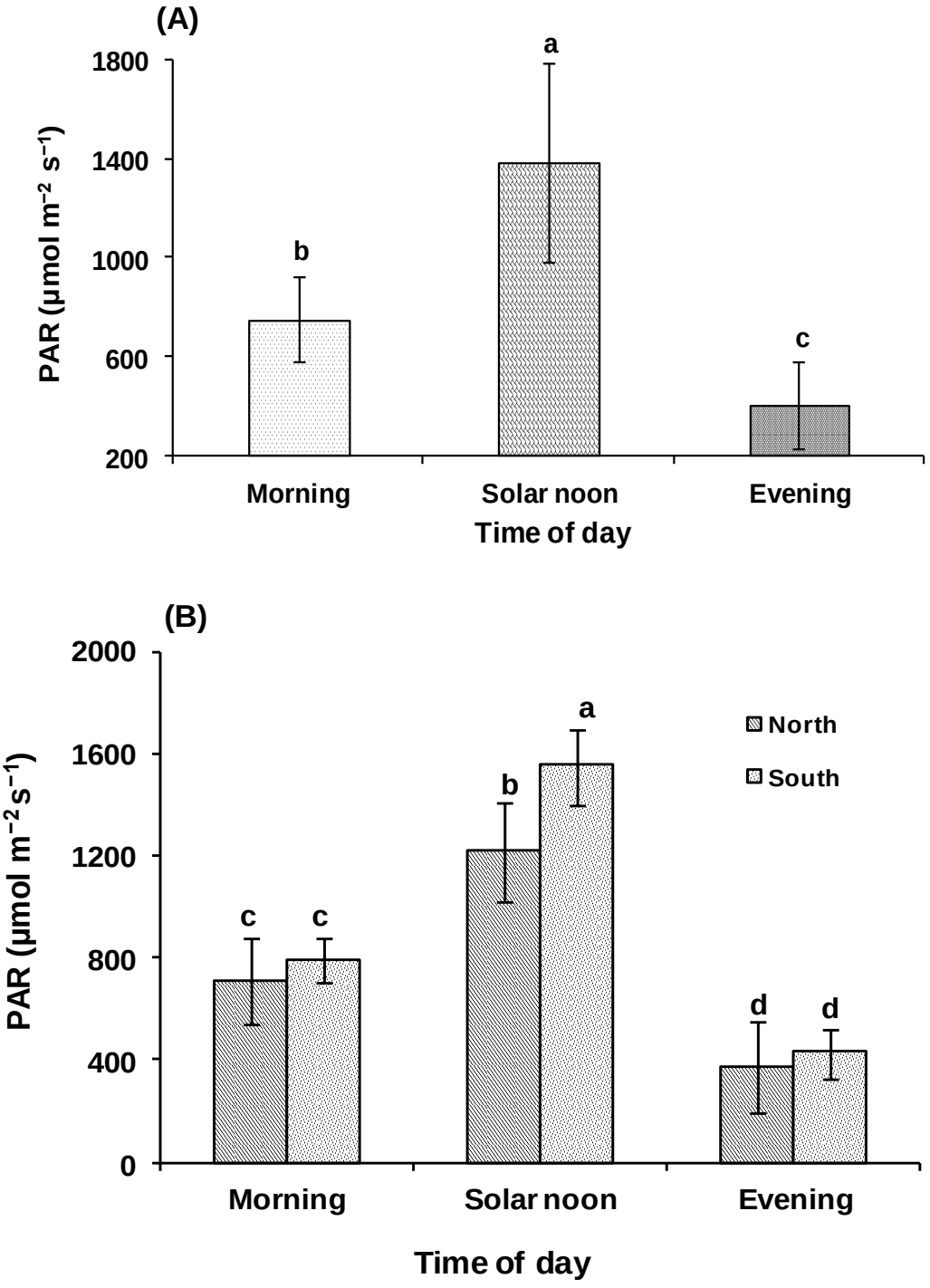

Fig. 5.6. Photosynthetically active radiation (PAR) (µmol m^{-2} s^{-1}) within Manitoba maple-oats alley cropping system as influenced by time of day (A) and time*orientation (B) (n = 90). Samples were taken three times per day (morning, solar noon, evening) bi-weekly two and half-month period within the oats plots at two orientations and three distances from the tree line. Means followed by same letters are not significantly different at $P \leq 0.05$ according to Tukey's HSD. (Morning = ca. 8:00, Solar noon = ca. 13:00 and Evening = ca. 18:00).

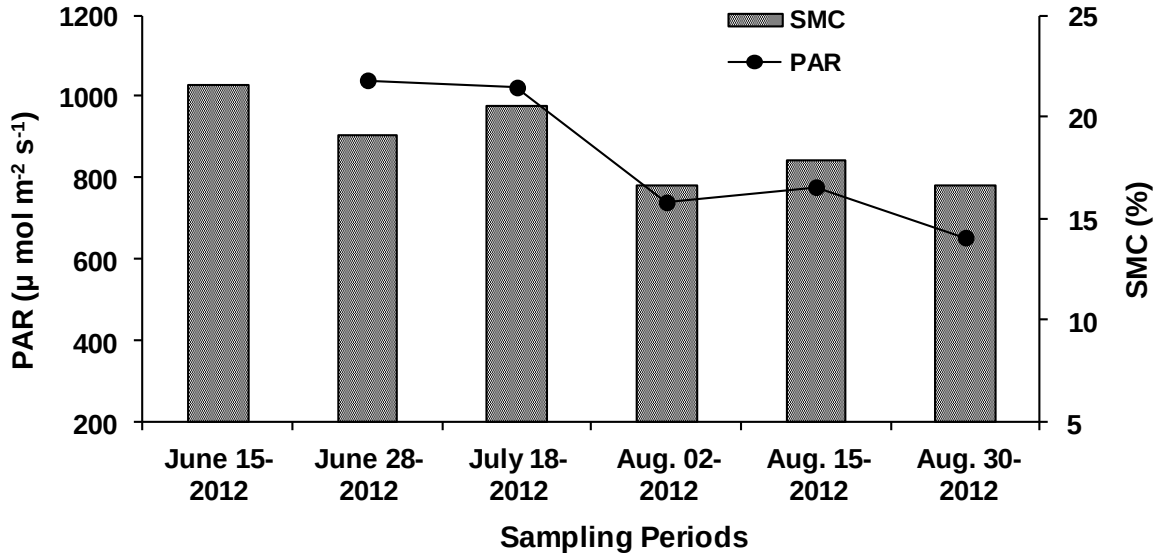

Fig. 5.7. Trends in gravimetric soil moisture content (SMC) and photosynthetically active radiation (PAR) (µmol m^{-2} s^{-1}) during the 2012 growing season as influenced by sampling period over the experimental period, in a Manitoba maple-oats alley cropping system in Indian Head, Saskatchewan, Canada.

5.5.3 Oats yield and forage quality

Oats DM content did not show significant differences due to the effects of distance, orientation and their interactions (Table 5.2). Fibre contents (NDF and ADF) were also not different due to the effects of distance, orientation and their combined interactions (Table 5.2). Despite the lack significance in oats DM content, ADF and NDF, the northern recorded higher mean values compared to the southern orientation (Table 5.2).

Total N and CP were affected by distance, orientation and their interactions (Table 5.2). Generally, the oats grown in northern orientation had higher mean values of TN and CP compared to that in the southern orientation (Table 5.2). Total N and CP in oats in the northern orientation were up to 27 % higher than those in the southern orientation. In the northern orientation, the TN and CP values at 2 m and 6 m, were similar and higher compared to corresponding values at 4 m. In the southern orientation, TN and CP generally increased with increasing distance from the tree rows (Table 5.2).

Table 5.2. Oats forage quality parameters in Manitoba maple-oats alley cropping system at Indian Head, SK. Plant samples (n = 30) were collected at the end of the growing season from the oats plots at three distances (2 m, 4 m and 6 m) from the tree line in the north and south of the tree row.

	Orientation				
			North Plot		
Distance (m)	**DM** §	**ADF** ¶	**NDF** #	**TN** ††	**CP** ‡‡
2	94.70 **a**‡ (0.18)†	38.97 **a** (0.87)	56.36 **a** (0.90)	1.68 **a** (0.12)	10.48 **a** (0.73)
4	94.64 **a** (0.56)	37.88 **a** (0.83)	59.02 **a** (1.33)	1.34 b**c** (0.08)	8.38 **bc** (0.52)
6	94.91 **a** (0.17)	38.00 **a** (2.58)	56.66 **a** (1.91)	1.62 **a** (0.05)	10.09 **a** (0.33)
			South Plot		
2	94.60 **a** (0.25)	35.86 **a** (1.07)	56.38 **a** (1.63)	1.23 **c** (0.06)	7.68 **c** (0.40)
4	94.55 **a** (0.15)	35.65 **a** (1.74)	54.49 **a** (1.93)	1.33 **bc** (0.04)	8.31 **bc** (0.29)
6	94.55 **a** (0.21)	35.44 **a** (3.54)	55.22 **a** (3.24)	1.53 **ab** (0.14)	9.54 **ab** (0.88)
			***P*- values**		
Distance (D)	0.607	0.905	0.906	**0.050**	**0.050**
Orientation (O)	0.128	0.084	0.203	**0.022**	**0.024**
D × O	0.557	0.968	0.462	**0.050**	**0.050**

† SEM, standard error of the mean

‡ Means (n = 30) in a column for each parameter followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD.

§ DM, dry matter content

¶ ADF, acid detergent fibre

NDF, neutral detergent fibre

†† TN, total nitrogen

‡‡ CP, crude protein

5.5.4. Trends in climate data (relative humidity and air temperature)

Due to malfunctioning of data loggers, only relative humidity (RH) and air temperature values from May up to the end of July, 2012 were retrieved and reported. Also, since no values for PAR and SMC were obtained in May (Fig. 5.7), to relate the climate data (Fig. 5.8) with PAR and SMC, only June and July RH and temperature were referred to in the discussion. The general trend for both RH and temperature were of increasing with progression into the summer period from the spring period (Fig. 5.8). Increasing RH and air temperature generally coincided with decreasing SMC and higher PAR (Fig. 5.7). The general trend in SMC was that of decreasing in average values with progression into the summer (i.e., July/August) from the spring period (May/June) and values range between 17-21 % (Fig. 5.7).

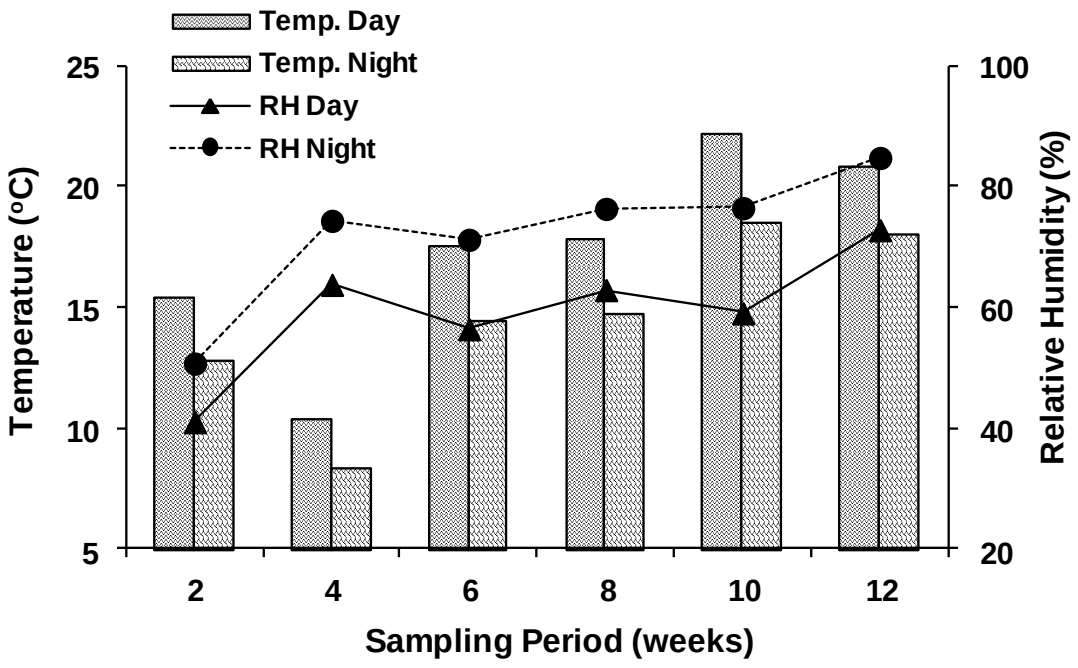

Fig. 5.8. Trends in diurnal and nocturnal air temperature (°C) and relative humidity (%) during the 2012 growing season on bi-weekly basis over the experimental period (May-July, 2012) across the Manitoba maple-oats alley cropping plots in Indian Head, SK, measured using USB-500 series data loggers. Diurnal = ca. 6:00 to 17:00 and nocturnal = ca. 18:00 to 5:00.

5.6 Discussion

5.6.1 Soil moisture content, photosynthetically active radiation and forage quality

The general trend of increasing SMC with distance from the tree row could be due to coincidental occurrence of both oats and Manitoba maple roots close to the tree row and corresponds to the zone at which the highest root length density occurs. This region coincides with the zone of competition for soil moisture and nutrients which is mostly exploited by both the tree and crop roots (Cardinael et al., 2012). This competition might have led to reduced concentration of tree and crop roots further away from the tree row, leading to reduced competition for available soil moisture (Moyer-Henry et al., 2006; Day et al., 2010). This trend was the assertion because despite the potential of tree tap-roots to develop deeper and further away from the tree, typically, most of the tree and crop roots in agroforestry system tend to concentrate in the top 30 cm of the soil profile and around the trees. This results in intense competition between trees and crops for soil moisture which can affect crop production (Jose et al., 2000a; Delate et al., 20005; Zamora et al., 2007). The reduced SMC in the south orientation might have been due to higher PAR which caused higher rate of evapo-transpiration from the soil and leaf surfaces, respectively, leading to lower DM content and forage quality parameters relative to the north orientation.

The lower yield of oats in the south orientation relative to the north orientation despite higher PAR proved the fact that there is no effect of PAR on yield of oats in this study. Oats plants close to the Manitoba maple row received lower PAR compared to those at the centre of the row and further away from the tree row. The non-significant effects of the tree on the yield of intercrop oats (C3 plant) shows that sun angle, despite being influenced by time of the day, tree height and orientation (Ding and Su, 2010) did not affect the DM, ADF and NDF with reference

to the proximity of the oats from the tree rows. The characteristics of the tree used in this study are that; it is relatively short, has broad-based and sparse canopy (personal observation). These characteristics may be the reason why it did not cast shadows on the intercrop in the alleys but allowed enough light to penetrate through the canopy to the northern orientation. Another possible reason for the non-significant effects of PAR on yield and nutrition in the present study may be explained by the fact that, the range of average PAR values recorded, i.e., ca. 400-1000 μmol m^{-2} s^{-1} were below the light saturation point of C3 plants of ca. 1,200 μmol m^{-2} s^{-1} or 65000 Lux (Nair, 1993). Hence, PAR could not have been the main determining factor for DM and forage quality. The fact that PAR did not have a major effect on yield and nutrition in oats in this study might be due to the fact that PAR becomes the major determining factor of biomass production only when belowground resources are not limiting (Zamora et al., 2008). This can also be due to the fact that oats being C3 is not too sensitive to light compared to C4 plants, as in a study where yield of two crops with different photosynthetic pathways i.e., corn (C4 plant) and soybean (C3 plant) as intercrops with two tree crops (hybrid poplar and silver maple) were compared. It was concluded from that study that, corn yield was negatively affected by the presence of the tree due to the reduction of light below the threshold value required by the corn (C4 plant) compared to the soybean (Reynolds et al., 2007). Once SMC was seen to play a major role in the south orientation, there was no indication of increased yield due to higher PAR, showing that competition for PAR did not play a major role in determining yield and nutrition of oats in this study. The finding from this study can be supported by that by Gillespie et al. (2000) who found that there is no correlation between PAR and photosynthetic rate in C3 plant as opposed to C4 plant (Gillespie et al., 2000).

Contrary to the expectation that, due to competition for SMC, PAR and nutrients at the tree-crop interface, there should be a reduction in DM content and other forage quality parameters within the zone of competition, the trend was different in this study. This can be explained by the trend in the average SMC in the top 0 to 20 cm within the zone of competition. This might have given optimum moisture to the intercrop to aid in proper growth because most of the roots of both species were concentrated within this region (Jose et al., 2004). Competition for SMC was more pronounced in the southern orientation compared to that in the northern orientation. The results from this study is similar to that of Jose et al. (2000a) who reported that competition for soil moisture reduced crop yield near black walnut. Although it is difficult to separate belowground competition for nutrients from that for soil moisture, it is widely accepted that competition for soil moisture is the dominant driving force in determining crop production in semi-arid regions (Jose et al., 2004). The results also supports that of studies that showed that crop productivity was reduced at the tree-crop interface, where competition for water is greatest (Newman et al., 1998; Jose et al., 2000a; Allen et al., 2004; Wanvestraut et al., 2004). The reported higher mean values of TN and CP in oats in the north orientation (up to 27 %) relative to that in the south orientation could be due to the competition for SMC. This conforms to the study conducted by Ding and Su (2010) who reported reduction in crop yield within the competition zone in Poplar (*Populus gansuensis*)-maize (*Zea mays* L.) agroforestry systems in China. There were consistent lower yields in maize (*Zea mays* L.) close to the poplar tree rows in a Poplar-maize alley cropping site adjacent the study site (personal observation). This may be due to competition for light or soil moisture. Overall, the results from this study did not support that of Reynolds et al. (2007) who reported that competition for light (PAR) was a factor in

soybean (*Glycine max.* L.) yield reduction in a multi-species temperate agroforestry system in southern Ontario, Canada.

In summary, due the widely accepted notion that competition for soil moisture is the dominant driving force in determining crop production in semi-arid regions, it can be concluded from this study that competition for water played a major role in determining yield and nutrition in oats, with effects of light (PAR) being minor. Proximity of oats crop to Manitoba maple tree row affected TN and CP, obvious at the south orientation more than the north orientation. The north orientation recorded a generally higher mean values in all the measured parameters but the south orientation recorded higher individual PAR values at all periods of the day.

6.0 SYNTHESIS AND CONCLUSIONS

The role that trees confer on associated crops when intimately integrated on the same land management unit, such as in agroforestry systems, has long been recognized (Nair, 1993). Nitrogen-fixing trees have the ability to fix atmospheric N_2 and subsequently transfer the fixed N_2 to the associated crops (Jensen, 1996; He et al., 2003). The practice of planting N_2-fixing trees with associated crops is common in the tropics as an N management strategy; however, in temperate agroforestry systems integrating N_2-fixing trees into land-use systems like alley cropping is rare (Nair et al., 1999). This is due to the abundance and historically low cost of N fertilizers and the low value of N_2-fixing trees (Nair et al., 1999).

Much of the earlier research in temperate systems looked at the role of these trees as windbreaks and shelterbelts (Kort, 1988). However, their role in alley cropping systems to supply N to associated crops has received little attention within the prairies. The role of trees in supplying N to associated crops represents an environmentally friendly means of introducing N into land use systems through the transfer of fixed N_2 (Paynel et al., 2008).

Integrating trees and crops on the same land management unit results in competition and/or facilitation for growth resources including soil moisture, nutrients and light. The balance between facilitation and / or competition for these resources at the tree-crop interface is the principal determinant of yield and productivity of intercropped species, especially the yield of associated crops (Nair, 1993). Exploiting the complementary or facilitative interactions between the woody and non-woody components and minimizing the competitive interaction is a key to the success of agroforestry systems (Ong et al., 1991; Ong, 1996).

Whereas the vast majority of research on N_2 fixation in the Canadian Prairies has focused on pulse crops (Stevenson et al., 1995; Kyei-Boahen, 2001; 2002), recent interest in some

agroforestry practices within the region has necessitated the quantification of N_2 fixation in trees. The research presented in this thesis addresses some of the knowledge gaps regarding the potential impacts of N_2-fixing trees in supplying N to associated crops, as well as, other modifications to growth resources at the tree-crop interface.

The general aim of this research was to determine the effects of trees in alley cropping systems on the yield and nutrition of forage crops through the supply of N to associated crops and to examine how the trees modify microclimatic conditions at the tree-crop interface. Nitrogen-15 dilution techniques were used to quantify N_2 fixation and N transferred to associated crops.

6.1 Summary of findings

Caragana, sea buckthorn and buffaloberry have being important trees in some land use systems within the Canadian Prairies since tree planting commenced; however, little research has been done to determine their role in supplying N. Estimates of % Ndfa in the test species were similar to those reported in the literature by Parrota et al. (1994) and Binkley (1997) who reported values between 48 and 100 % for a variety of temperate zone tree species. In the present study, the ^{15}N isotope dilution experiment provided more reliable estimates of % Ndfa than the ^{15}N natural abundance experiment because of more uniform nodulation. However, care must be taken in making firm conclusions, as the applicability of the method under field conditions can be problematic. Specifically, because trees were grown in containers, it was possible to uniformly label the soil with the applied ^{15}N-fertilizer; a condition unlikely to apply in a field situation.

Under greenhouse conditions, the greatest amount of N_2 was fixed by caragana (73 kg N $ha^{-1}yr^{-1}$) followed by sea buckthorn (67 kg N $ha^{-1}yr^{-1}$) and then buffaloberry (16 kg N $ha^{-1}yr^{-1}$) (Chapter 3). The range of N_2 fixed values were generally within the range of BNF potentials of selected tree species for temperate agroforestry systems, i.e. 20 to 500 kg N $ha^{-1}yr^{-1}$ for leguminous trees or shrubs like caragana and 24 to 236 kg N $ha^{-1}yr^{-1}$ for non-leguminous trees and shrubs, like sea buckthorn and buffaloberry (cited in Jose et al., 2004). Caragana and sea buckthorn fixed significant amounts of N_2, capable of meeting the requirement of forage and cool-season crops within Saskatchewan. The higher contribution of caragana to total amounts of N_2 fixed also support the hypothesis that caragana would have a higher BNF compared to sea buckthorn and buffaloberry and may be a proof that the legume-*Rhizobium* association makes a greater quantitative contribution to N cycle than any other associations (Herridge et al., 2008).

Another objective of the research was to evaluate the transfer of fixed N_2 to associated crops. In doing so it can show the possibility of the test trees meeting the N requirements of forage and cool-season crops when integrated intimately under the same land management unit. Nitrogen transfer from caragana to triticale and oats were up to 64 % and 70 %, respectively in the 2011 and 2012 growing seasons. This contributed up to 32 kg N ha^{-1} and 23 kg N ha^{-1} per growing season for triticale and oats, respectively. This shows that up to 43 % of the fixed N_2 in caragana can be transferred to the cereal crop after one growing season. This falls in the range of reported values of N from N_2-fixing to non-N_2-fixing companion crops of 0 to 68 % of the N content in the companion crop (Høgh-Jensen and Schjoerring, 1994; Rasmussen et al., 2007). The amount of N from caragana transferred to triticale (43 %) and oats (32 %) in the 2011 and 2012 growing season after one growing season were higher than estimated amount of N transfer

from legume tree *Gliricidia sepium* to fodder grass *Dichantium aristatum* (14 %) (Jalonen et al., 2009) and from *Acacia senegal* to wheat (*Triticum turgidum* durum) (14 %) (Isaac et al., 2012).

Overall, while it is clear that interspecific competition was present in the alley cropping systems, the high rate of BNF in the test species (Chapter 3) and the substantial rate of N transfer to associated triticale and oats (Chapter 4) suggest that N was not a limiting resource in the caragana-triticale and/or oats systems.

Effective management of competition and/or facilitation is essential to improving yield and nutrition of associated species in alley cropping systems (Ong et al., 1991). Ideally this could be done in a manner that minimizes the deleterious effects of competition while retaining the environmental and facilitative or complementary benefits of alley cropping systems (Harrington et al., 2003; Wanvestraut et al., 2004; Zamora et al., 2009). However, the practice of alley cropping is not a widespread practice in Saskatchewan. Therefore, there is dearth of information on the impacts of biophysical interactions on the yields and nutrition of especially the crop component. In Chapter 5, I evaluated the effects of competition for SMC and PAR between Manitoba maple and oats at the tree-crop interface. From the experiment, it was evident that as it will be the case in a typical semi-arid climate like Saskatchewan, competition for SMC was the main determining factor in yield and nutrition in oats (Ong et al., 1991; Chirko et al., 1996). The non-significant effects of PAR on forage quality indices in this study can be explained by the fact that the average PAR values were up to 1,000 μmol m^{-2} s^{-1}, close to the light saturation point of C3 plants (1,200 μmol m^{-2} s^{-1}) (Nair, 1993). Despite the differences in forage quality indices due to soil moisture competition, which were lower on the south side of the Manitoba maple tree relative to the north side, alley cropping system might be an option in areas where there is scarcity of arable land. Under those circumstances, the overall productivity of the components

from the alley cropping systems may be higher compared to the productivity of the individual components grown in a monoculture system.

6.2 Recommendations and Future Research

In areas where arable land is scarce due to urbanization or other constraints, alley cropping systems involving N_2-fixing trees can be adopted as means to increase productivity per unit land area through replenishing soil fertility. This may provide multiple outputs from the tree and crop components to farmers and landowners. Due to the efficient and substantial amount of N_2 fixed by the test species, the species can be incorporated into land-use systems with either inherently low soil fertility or with restrictions on the use of synthetic N fertilizers such as in organic agriculture. The trees in those systems can serve as sources of available N and other nutrients. The substantial amount of N transfer from the trees to the crop even in wider alleys (15 m) shows that the trees can meet the N requirements of the associated crops. This idea can be exploited as a means to replace or complement the use of synthetic N fertilizers while permitting smooth operation of farm machinery. Finally, despite the competition for soil moisture between trees and crops, the system has the potential to yield higher overall output compared to the individual outputs of the components in a monoculture.

In this thesis, the BNF potential of the test species, their contribution to meeting the N requirements of cool-season crops through N transfer as well as interspecific competition and/or facilitation in alley cropping systems under the climatic conditions of Saskatchewan have been elucidated. However, as a next step, the following questions need further research.

The experiment on quantifying BNF in the test species was one of the few research works to determine BNF in the test species. It was limited in duration due to my Masters program and

confounding problems of seedlings being "pot-bound" if allowed to grow for a longer time, so effects of time and other parameters could not be assessed. Therefore, a long-term study, possibly in the field should be undertaken to determine the effects of time and other biotic and abiotic parameters on BNF in the test species, and to corroborate and extend the conclusions drawn from this study.

The N transfer experiment provided results from two successive growing seasons on the effects of N on nutritive value in triticale and oats and showed that the N can meet the requirements of cool-season crops. It was concluded that, the transfer pathway was chiefly belowground through varied pathways. The study was not; however, able to delve into which particular belowground pathway (s) was/were dominant. Therefore, as a further step, the contributions of each belowground mechanism should be assessed to know their respective inputs at each stage in the growth cycle of the associated species. Other factors affecting N transfer should also be assessed in future studies, as a recent study by Isaac et al. (2012) highlights the effects of phosphorus levels on N transfer. Finally, there should be further probing into the nature of the transfer, that is whether unidirectional or bidirectional and whether there is concurrent C transfer with the N transfer.

To confirm the findings from the effects of biophysical interactions for soil moisture and light on yield and nutrition of forage crops, a longer-term study should be conducted at least over two growing seasons. Also, relative humidity and air temperature data should be collected on per plot basis as opposed to around the entire experimental site to determine their actual impacts on yield and nutrition.

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APPENDIX A

Table A.1. ANOVA table on the effects of different factors on gravimetric soil moisture content (%) collected bi-weekly in three depths at two orientations in a Manitoba maple-oats alley cropping site at Indian Head, SK.

Effect	Num. DF	Den. DF	F Value	Pr > F
Week	5	416	31.42	<.0001
Distance	2	416	1.07	0.3445
Orientation	1	416	26.15	<.0001
Depth	2	416	16.97	<.0001
Distance*Orient.	2	416	2.01	0.1359

Table A.2. ANOVA table showing mean separations using Tukey`s HSD of different factors on gravimetric soil moisture content (%) collected bi-weekly in three depths at two orientations in a Manitoba maple-oats alley cropping site at Indian Head, SK.

Week	Distance	Orientation	Depth	Estimate	Standard error	Letter Group
2	_			21.5738	0.4733	**a**
4	_			19.1019	0.4733	**bc**
6	_			20.4818	0.4733	**ab**
8	_			16.5719	0.4733	**d**
10	_			17.8532	0.4733	**cd**
12	_			16.6172	0.4733	**d**
_	2			18.3924	0.3957	**a**
_	4			18.8860	0.3957	**a**
_	6			18.8216	0.3957	**a**
_	_	N		19.4665	0.3662	**a**
_	_	S		17.9335	0.3662	**b**
_	_		0-20	19.7854	0.3957	**a**
_	_		20-40	18.6676	0.3957	**b**
_	_		40-60	17.6469	0.3957	**c**

APPENDIX B

Table B.1. ANOVA table on the effects of different factors on photosynthetically active radiation content taken bi-weekly at three times of the day at two orientations in a Manitoba maple-oats alley cropping site at Indian Head, SK.

Effect	Num. DF	Den. DF	F Value	Pr > F
Week	4	342	35.21	<.0001
Distance	2	342	67.01	<.0001
Orientation	1	342	34.75	<.0001
Time of day	2	342	471.30	<.0001
Distance*Orien.	2	342	42.59	<.0001
Orientation*Time	2	342	11.49	<.0001

Table B.2. ANOVA table showing mean separations using Tukey`s HSD of different factors on PAR taken bi-weekly at three times of the day at two orientations in a Manitoba maple-oats alley cropping site at Indian Head, SK.

Week	Distance	Orientation	Time of day	Estimate	Standard error	Letter Group
2	_			1040.45	41.9927	**a**
4	_			1022.77	41.9927	**a**
6	_			739.66	42.1441	**bc**
8	_			772.88	41.9927	**b**
10	_			652.75	41.9927	**c**
_	2			628.29	37.6043	**b**
_	4			956.68	37.6043	**a**
_	6			951.71	37.6652	**a**
_	_	N		767.69	35.2345	**b**
_	_	S		923.72	35.2056	**a**
_	_		Morning	750.47	37.6043	**b**
_	_		Afternoon	1384.23	37.6043	**a**
			Evening	404.97	38.6652	**c**
__	__					

APPENDIX C

Table C.1. Isotopic signatures in roots, shoots and whole plants in buffaloberry, caragana, sea buckthorn and a reference species (choke cherry) under the greenhouse condition 120 DAP by the natural abundance ($\delta^{15}N$) and ^{15}N dilution (^{15}N) techniques.

Plant components	Buffaloberry	Caragana	Sea buckthorn	Choke Cherry
		$\delta^{15}N$ by Natural Abundance †		
Root	4.050 (nd)	4.000 ‡ (0.2612) §	1.283 (0.3076)	5.683 (0.0536)
Shoot	5.470 (nd)	2.520 (0.5172)	1.795 (0.7178)	6.077 (0.1891)
Whole Plant	9.635 (nd #)	7.240 (0.6424)	4.220 (0.7200)	11.903 (7.1100)
		^{15}N by ^{15}N Dilution ¶		
Root	0.422 (0.0067)	0.392 (0.0034)	0.4077 (0.0010)	0.461 (0.0077)
Shoot	0.425 (0.0116)	0.402 (0.0034)	0.393 (0.0017)	0.500 (0.0031)
Whole Plant	0.779 (0.0671)	1.006 (0.0686)	0.678 (0.0385)	1.319 (0.0968)

† Values are calculated based on one replicated for buffaloberry (n = 1) and four replicates (n = 4) each for caragana, sea buckthorn and choke cherry

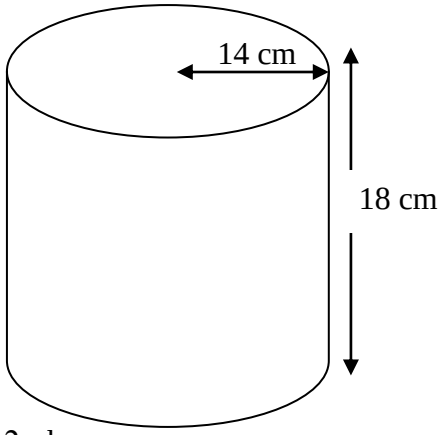
‡ Values are calculated based on six replicates (n = 6) each for buffaloberry, caragana, sea buckthorn and choke cherry

§ Values in parenthesis represent the standard error of the mean (SEM)

nd means no data as only one replicate was used

APPENDIX D

Up-scaling whole plant amounts of N_2 fixed in g tree^{-1} to N_2 fixed in kg N ha^{-1} yr^{-1} in buffaloberry, caragana and sea buckthorn under greenhouse condition 120 DAP by the natural abundance ($\delta^{15}N$) and ^{15}N dilution (^{15}N) techniques.



Surface area of the pot $= 2\pi r^2 + 2\pi rh$

$= 2\pi (0.14m)^2 + 2\pi (0.14m)(0.18m)$

$= 0.123166m^2 + 0.1583568m^2$

$= 0.2815232m^2$

Relating the area of the pot to area of one hectare

$= 0.2815232m^2$ is equivalent to 10,000 m^2

From this relationship, the amount of N_2 fixed in g tree^{-1} in the pot was converted to amount of N in kg ha^{-1}. Taking the growing period of the experiment to be 120 days for natural abundance samples and 90 days for the ^{15}N dilution samples, the values were converted to represent kg N ha^{-1} yr^{-1}, assuming the thaw-freeze period is ca. 160 days.

Based on this assumption, the amount of N_2 fixed on whole plant basis in kg N ha^{-1} yr^{-1} from those in g tree^{-1} per 90 and 120 days for ^{15}N dilution and natural abundance, respectively (Fig. 3.4 B and 3.4 A) are as below:

	Natural abundance (kg N ha^{-1} yr^{-1}**)**	**^{15}N dilution (**kg N ha^{-1} yr^{-1}**)**
Buffaloberry	nd	16
Caragana	15	73
Sea buckthorn	11	67